RESPIRATORY SYNCYTIAL VIRUS (RSV) VACCINES ON THE HORIZON: DATA TO INFORM CLINICAL TRIALS AND IMPLEMENTATION

by

Jessica E. Atwell, MPH

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Abstract

Respiratory syncytial virus is the leading cause of viral lower respiratory tract infection (LRI) globally, resulting in an estimated 33.8 million cases and 66,000 to 234,000 deaths each year. The majority of deaths occur in young infants, predominantly those in low and middle-income countries where oxygen therapy is unavailable or inadequate.

After decades of development, vaccines for RSV are on the horizon. More than 50 candidate vaccines are in clinical development and as of early 2016, 9 were in clinical trials. However, important questions pertaining to the evaluation and implementation of such vaccines remain. This dissertation is an attempt at answering three such questions:

1) Are placental malaria and hypergammaglobulinemia associated with impairment of transplacental transfer of RSV antibodies?

2) Which respiratory viruses predominate among children with pneumonia in Papua New Guinea, a tropical equatorial setting?

3) What objective clinical signs and symptoms are predictive of life-threatening RSV LRI and what case definitions might be suitable for use as endpoints in efficacy trials for pediatric and maternal RSV vaccines?

We measured naturally-acquired RSV antibody levels in 300 paired maternal and cord blood specimens from two temporally distinct cohorts in an area of Papua New Guinea (PNG) endemic for malaria. We also followed 341 children in this same region of PNG from birth to age 2 years via active and passive surveillance for respiratory infections, and tested nasopharyngeal specimens with a panel of 21 respiratory viruses. We then evaluated a

dataset of children hospitalized with RSV in and around Buenos Aires, Argentina to determine which signs and symptoms were predictive of life-threatening disease.

We found that placental malaria is not associated with impaired transplacental transfer of RSV antibody, but that hypergammaglobulinemia is. Rhinovirus, adenovirus, bocavirus and RSV predominate among children with LRI in PNG, co-infections are common, and RSV contributes substantially to severe pneumonia. Oxygen saturation measured via pulse oximetry ≤90, tachypnea and tachycardia are positively associated with critical RSV LRI, and when measured at hospital admittance, may be useful as predictors of severity in the context of a clinical trial.

Thesis Advisor:

Ruth Karron, MD
Professor, Department of International Health

Thesis readers:

Marie Diener-West, PhD
Professor, Department of Biostatistics

Bill Moss, MD
Professor, Department of Epidemiology

Laura Hammitt, MD
Assistant Professor, Department of International Health

Alternate thesis readers:

Joanne Katz, ScD
Professor, Department of International Health

William Checkley, MD
Assistant Scientist, School of Medicine

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~

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Abbreviations

Ab	Antibody
ALRI	Acute lower respiratory tract infection
ANC	Antenatal care
AUC	Area under the curve
CI	Confidence interval
CMTR	Cord to maternal titer ratio
CWRU	Case Western Reserve University
ELISA	Enzyme-linked immunosorbent assay
FcRn	Neonatal Fc receptor
FIS	Fetal immunity to malaria study
GA	Gestational Age
GM	Geometric mean
Hib	*Haemophilus influenzae* type B
HIV	Human Immunodeficiency Virus
HR	Heart rate
IFNs	Interferons
IgG	Immunoglobulin G
IPTp	Intermittent preventive treatment of malaria in pregnancy
IUGR	Intrauterine growth restriction
LBW	Low birth weight
LCWI	Lower chest wall indrawing
LMIC	Lower and middle income countries
LRI	Lower respiratory tract illness
NP	Nasopharyngeal
OR	Odds ratio
PERCH	Pneumonia Etiology Research for Child Health Study
PM	Placental malaria
PNG	Papua New Guinea
PNG IMR	Papua New Guinea Institute for Medical Rearch
PRN	Plaque reduction neutralization
PRNT	Plaque reduction neutralization titer
RNA	Ribonucleic Acid
ROC	Receiver operator curve
RR	Respiratory rate
RSV	Respiratory Syncytial Virus
RSV IGIV	Respiratory Syncytial Virus immune globulin intravenous (RespiGam)
SpO_2	Oxygen saturation
STB	Syncytiotrophoblast
URI	Upper respiratory tract infection

Chapter 1: Introduction

1.1 Respiratory Syncytial Virus

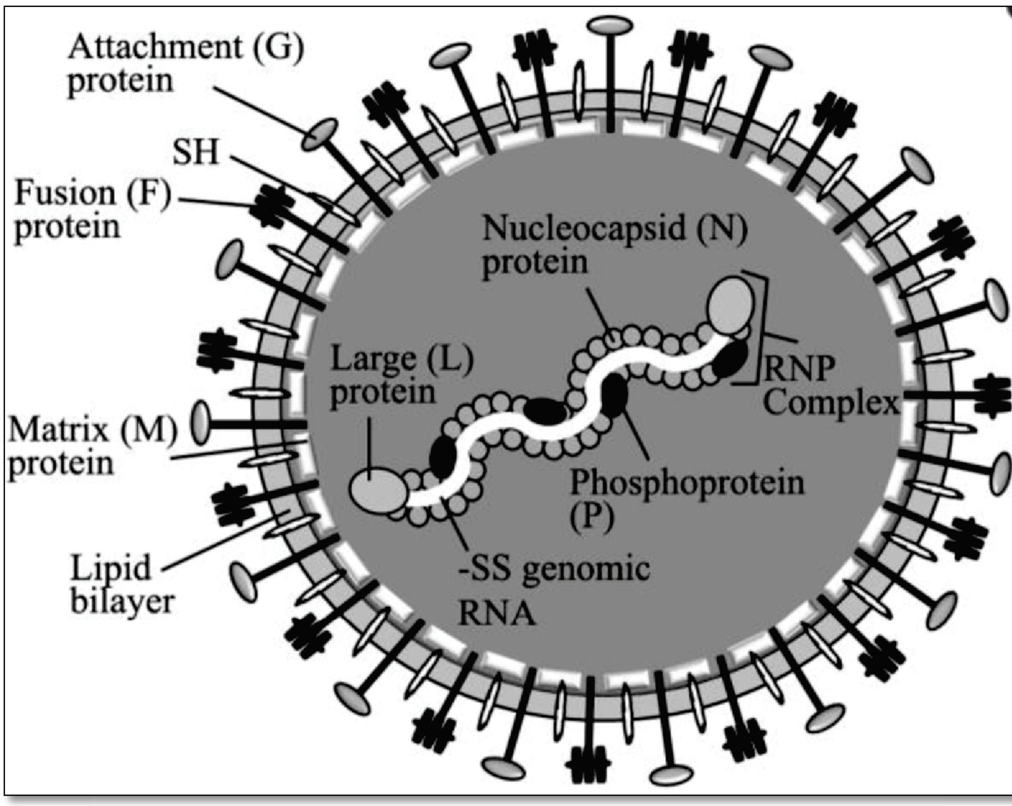
1.1.1 The Virus

Respiratory syncytial virus (RSV) is non-segmented, negative sense, single stranded RNA virus and a member of the Paramyxoviridae family, subfamily pneumovirinae, genus pneumovirus.[1] The virus encodes 10 proteins in total: three transmembrane surface proteins (F, G, and SH), two matrix proteins (M and M2), three nucleocapsid proteins (N, P and L), and two non-structural proteins (NS1 and NS2).[1] RSV neutralizing antibody (Ab) is induced to only two of the proteins, predominantly F, but also G, which along with transmembrane protein SH are responsible for fusion of the viral envelope to the host cell membrane. The NS1 and NS2 proteins are important in viral pathogenesis, both in altering the host's innate immune response to the virus via disruption of type I and type II interferons (IFNs) and contributing to airway obstruction by promoting the sloughing of infected epithelial cells within the respiratory tract. [1]

RSV strains can be classified via cross-neutralization into two groups, A and B. Some data suggest group B infection may be less severe than group A, but this is incompletely understood. Infection with RSV does not result in life-long immunity from homologous or heterologous groups, and children are often re-infected with virus of the same group.[1-3]

In 1956, J.A. Morris and colleagues at Walter Reed Army Institute of Research first isolated a virus they called chimpanzee coryza agent from chimpanzees with upper respiratory illness (URI). Later that year, the same virus was isolated from infants with pneumonia and croup by Robert Chanock and colleagues at Johns Hopkins Hospital.[4-6] It was renamed respiratory syncytial virus, after the syncytia formed by multiple infected cells in cell culture.[4, 5] **Figure 1.1** is an illustration of RSV.[7]

Figure 1.1: Respiratory Syncytial Virus (Al Johani, et al)



1.1.2 Epidemiology of RSV

RSV is the leading cause of viral acute lower respiratory tract illness (LRI) in infants and children worldwide. Almost all children will experience an RSV infection at least once by their second birthday; half of children will have been infected twice. [1, 8]

New estimates of the global burden of RSV LRI are in progress, but a review conducted in 2005 estimated an annual burden of 33.8 million cases of RSV in children under five (95% CI 19.3-46.2). Estimates of annual RSV-associated mortality range from 66,000 to 234,000.[9, 10]

Re-infection can occur throughout life, and although frequently symptomatic, LRI in healthy adults and healthy older children is rare.[8] While historically considered a disease of pediatric importance, there is a growing appreciation of the burden of RSV in older adults. Recent studies suggest nearly 180,000 RSV-associated hospitalizations each year in the US among the elderly and 11,000 deaths.[11-13]

In temperate climates, RSV is most prevalent from late fall through early spring, causing annual epidemics. In tropical settings, the seasonality generally coincides with the rainy season.[14, 15] However, there is a paucity of data relating to RSV burden, seasonality, and contribution to LRI in tropical and non-African settings. [See objective 2].

RSV is a human-only pathogen, and is spread via large droplet respiratory secretions, necessitating close contact with infected individuals or contaminated surfaces for transmission. The virus can survive on fomites for several hours, which facilitates nosocomial transmission.[16-21]

Mortality is highest in settings where oxygen therapy and supportive care are inadequate or unavailable; therefore, deaths occur disproportionately in low and middle-income countries (LMIC).[10, 22] However, there is a tremendous economic burden of RSV in high-income countries.[22-25] In the US alone, direct medical costs of bronchiolitis caused by RSV infection are estimated to exceed $543 million dollars annually.[25]

1.1.3 Clinical presentation of RSV

RSV disease can vary in severity and presentation, ranging from mild URI to life-threatening bronchiolitis and pneumonia.[1] RSV is also the most frequent cause of viral otitis media in children.[1, 16, 26, 27]

Acute illness generally lasts 5-10 days, although lingering cough is common. The infection begins with mild upper respiratory signs, cough and low-grade fever, progressing into lower respiratory tract involvement, worsening cough, increased respiratory rate, and chest wall indrawing.[16] Crackles and wheeze may be heard on auscultation, and radiologic findings often show hyperinflation, peribronchial thickening, and areas of atelectasis.[16] In very young infants (<6 weeks old), poor feeding, lethargy and apnea may occur without other respiratory symptoms.[28]

Conditions such as premature birth, congenital heart defects, Down syndrome, chronic lung disease, and immunodeficiency can increase risk of complications. [29-32] However, the majority of infants with severe RSV disease are full-term and healthy.[33] Infants under three months old are the most severely affected.[16]

In healthy young adults, mild URI is typical of RSV infection, although up to one quarter may experience lower respiratory involvement and persons with immune suppression may experience more severe disease.[1, 16] RSV has historically been underappreciated in the elderly, but advancements in molecular diagnostics have helped improve its recognition as a pathogen of importance.[34] In the elderly, both upper and lower respiratory involvement is common, with 8-13% of patients experiencing respiratory failure and 2-5% of cases resulting in death.[13] Disease may be more severe among those with existing lung disease (such as chronic obstructive pulmonary disease) or congestive heart failure.[16] Due to the contagious nature of RSV, it can become particularly problematic in hospitalized settings or in communal living facilities, such as nursing homes or assisted living facilities.[11-13, 35]

1.1.4 Long-term morbidity

Severe RSV infection in early life may lead to asthma and other manifestations of long-term lung dysfunction, as up to half of infants hospitalized with RSV will exhibit recurrent wheezing or other long-term sequelae.[1, 16] These links have yet to be definitively established, but this is an area of active research. An alternative hypotheses is that children genetically predisposed to develop asthma may also be predisposed to severe RSV illness at a young age, however these hypotheses are not necessarily mutually exclusive: both explanations may be possible. [1, 5, 16, 36]

1.1.5 Immunity to RSV

Natural immunity to RSV is incomplete, as re-infection occurs throughout life.[37] The RSV F glycoprotein may stimulate innate immune responses via Toll-like receptors and CD14.[38] Cell-mediated (cytotoxic CD8+ T-cells) and humoral immunity (serum and secretory antibody) have been shown to develop after infection and likely contribute to protection from LRI in non-naïve, healthy older children and adults. [1] Correlates of protection for RSV are not completely understood nor clearly defined, but high levels of serum neutralizing antibody (as measured using plaque-reduction neutralization assays) have been associated with protection of the lower respiratory tract, and passively acquired RSV-neutralizing antibody can protect infants against RSV LRI.[8, 39-43]

1.1.6 Treatment and prevention of RSV

Despite the burden of RSV and recognition of it as an important pediatric pathogen for many decades, there are no currently licensed vaccines for RSV. Treatment in middle and high-income countries is generally only supportive, consisting predominately of oxygen therapy.

Because RSV neutralizing antibody can protect against LRI, monoclonal antibody therapies were developed and are currently available for prevention of RSV in high-risk infants. Palivizumab (also known as Synagis, developed by MedImmune), a humanized mouse monoclonal antibody directed against RSV F, was licensed in 1998 and has been shown to reduce RSV-related hospitalization of high-risk infants by nearly half.[44]

Guidelines vary by country, but as of late 2015, the American Academy of Pediatrics recommends that palivizumab be administered via injection monthly for a maximum of five months to infants with certain chronic illnesses including chronic lung disease of prematurity or congenital heart disease, or to infants born at a gestational age of 29 weeks or less who are 12 months or younger at the start of the RSV season.[45] Unfortunately, palivizumab is expensive, and while cost-effective for preventing hospital admissions for specific subgroups, it remains prohibitively expensive for use more broadly or in LMIC where the burden of RSV mortality is greatest. Efforts are underway to develop similar prophylactic products (i.e. MEDI8897, NCT02290340) that may require fewer doses and or may be less expensive. Several other small molecules and antiviral treatments are also in the early stages of clinical development. [46-48]

1.2 Vaccine development

1.2.1 History and challenges

Vaccine development has been hindered by gaps in understanding of RSV immunity and pathogenesis, the difficulty of developing vaccines for use in very young infants, and the unfortunate legacy of early vaccine trials conducted in the late 1960s in the U.S.

In 1969, a formalin-inactivated RSV vaccine candidate resulted in enhanced disease in vaccinated children who encountered wild type RSV in the subsequent RSV season. Many vaccinees were hospitalized and two children died. [49, 50] It remained unclear for decades precisely why this occurred, but recent studies using data from recipients of the formalin-inactivated vaccine suggest that it may have stimulated production of non-protective, low avidity antibody that upon exposure to RSV led to enhanced disease by failing to neutralize wild type virus, promoting unrestricted activation and stimulation of T-cells primed by the vaccine, and immune complex formation in infected tissues. [51]

Despite these setbacks, there has been continued interest in RSV among a relatively small field of dedicated researchers, and with recent advancements in virology and immunology, there is renewed optimism in vaccine development. As of early 2016 there are more than 50 candidate vaccines in development, including 12 in clinical trials. One vaccine, a maternal immunization candidate (NCT02624947), began phase III testing in late 2015.[52, 53]

Vaccine development is focused on four main target populations: 1) very young infants, 2) older infants and children, 3) pregnant women, and 4) the elderly. It is likely that multiple vaccine types will be needed to protect all high-risk groups. [54-56] For infants and children, both passive (maternal vaccines) and active immunization strategies are being pursued. With peak disease occurring in very early infancy, maternal immunization may be particularly helpful in improving immunity when risk of complications and death is greatest, and perhaps especially in settings where supportive care of infants is not available. However, reinfection and a substantial burden in older children (beyond the protective period of transient maternal antibodies) demonstrate a need for active immunization of older infants and/or young children as well. With the growing recognition of the burden of RSV in the elderly comes the realization that adults may also benefit from improved protection from severe RSV. [54-56]

Sterilizing immunity is not expected for any RSV vaccines, therefore the primary goal of all vaccines will be to protect against RSV-associated LRI.[1, 56, 57] This poses some challenges for clinical development, particularly for determination of efficacy in clinical trials of vaccines for both active and passive use. There is a need for RSV vaccine developers, clinical investigators, and regulators to decide on standardizable and objective case definitions for clinically relevant and vaccine-preventable RSV disease. Furthermore, there is a need to determine whether certain clinical signs, symptoms, or parameters (such as oxygen saturation measured by pulse oximetry—SpO_2) can help differentiate between children whose RSV LRI will be self-limited and those in whom RSV LRI will sufficiently severe that mortality would be likely in the absence of costly and globally scarce interventions, such as assisted ventilation.[58] [See objective 3]

1.2.2 Vaccines in development

Several types of RSV vaccines are in development, including attenuated, whole inactivated, particle-based, subunit, nucleic acid and gene-based vector vaccines. The current development pipeline is succinctly described in the PATH RSV Vaccine Snapshot resource (http://www.path.org/vaccineresources/details.php?i=1562; Appendix 1).

As different vaccine types are better suited for different target populations, research is focusing on non-replicating vaccines for passive protection of very young infants via maternal immunization, and replicating vaccines (either live-attenuated or vectored) for use in older infants and young children.[1, 54-56, 58] Non-replicating vaccines might also be of use in non RSV-naïve children and older adults.[1, 54-56]

Two of the products furthest on the development pipeline are based on the RSV F protein (the target of RSV-neutralizing antibody produced after natural infection, the tertiary structure of which has only recently been solved [59-63]); one is a nanoparticle vaccine (Novavax, NCT02624947)[64, 65] that entered phase III trial in pregnant women in late 2015; the other a pre-fusion F subunit vaccine (GlaxoSmithKline, NCT01905215, NCT02360475) that has been well-tolerated and immunogenic in healthy men, and is currently being evaluated in healthy women (phase II).

Live-attenuated vaccines for use in older infants are also showing promise, including one live-attenuated vaccine made from virus lacking the entire sequence responsible for coding the M2-2 protein (rather than just a point mutation). [66] This deletion mutant is substantially restricted in replication, but exhibits an up-regulation of gene transcription, leading to increased immunogenicity and strong neutralizing antibody responses.[66] Surveillance conducted in vaccine and placebo recipients in the RSV season following vaccine administration suggests protection against wild-type disease, but this will require further study.[66] Not only does this candidate appear to navigate the trade off between attenuation and immunogenicity that often complicates the design of live-attenuated vaccines, it may also prime for an anamnestic response following natural RSV infection.[66] Studies with this vaccine are ongoing.

1.3 Maternal immunization

1.3.1 Opportunities and challenges

Maternal immunization for protection of young infants against RSV is a promising strategy, yet there are also challenges to this approach. As opposed to active or direct immunization of an individual to produce humoral and cellular immunity, maternal immunization is a passive form of protection for the at-risk infant. Pregnant women or women of childbearing age are immunized to increase their production of specific antibody. Those antibodies are then actively transported across the maternal-fetal interface of the placenta (see below), where they can protect the infant from disease in the first few weeks and months of life.

Maternal immunization has been used successfully since the 1970s for reduction of neonatal tetanus.[67] The success of this program encouraged exploration of maternal immunization for passive protection of infants from other diseases, most notably influenza and pertussis. Vaccines for both are currently recommended by the ACIP for use in pregnant women. Maternal immunization is also being explored for pathogens that do not yet have licensed vaccines: most notably group B streptococcus and RSV.[68] Interestingly, candidate vaccines for these pathogens are being designed and tested explicitly for maternal immunization, and if effective, will seek licensure specifically for that indication. This is in contrast to vaccines for influenza and pertussis, which sought approval and recommendation for use in pregnant women after initial licensure.

The greatest risk for severe RSV is in early infancy, but vaccine response in very young infants is often poor and achieving protective titers via active immunization generally takes multiple doses of vaccine given throughout the first months of life.[1] As with tetanus and pertussis, protection against RSV is needed immediately after birth. Studies have shown that maternal antibodies for RSV are actively transported across the placenta[40, 69-72] and that passively acquired RSV neutralizing antibody protects infants from RSV LRI.[41, 45] Maternal immunization could provide an important bridge of protection between birth and active immunization of older children. There are several RSV subunit vaccines in development for use in maternal immunization as described above.

1.3.2 Transplacental transfer of antibody

The success of maternal immunization is predicated on efficient transplacental transport of antibodies. Maternal IgG antibodies are actively transported across the placenta to the fetus via binding to the IgG-transporting neonatal Fc receptor (FcRn), beginning in the second trimester and continuing until delivery.[73] Substantial transfer of antibody requires adequate levels of pathogen-specific immunoglobulin G (IgG) in the mother's blood during the time of transfer, term delivery, and a functionally intact placenta.

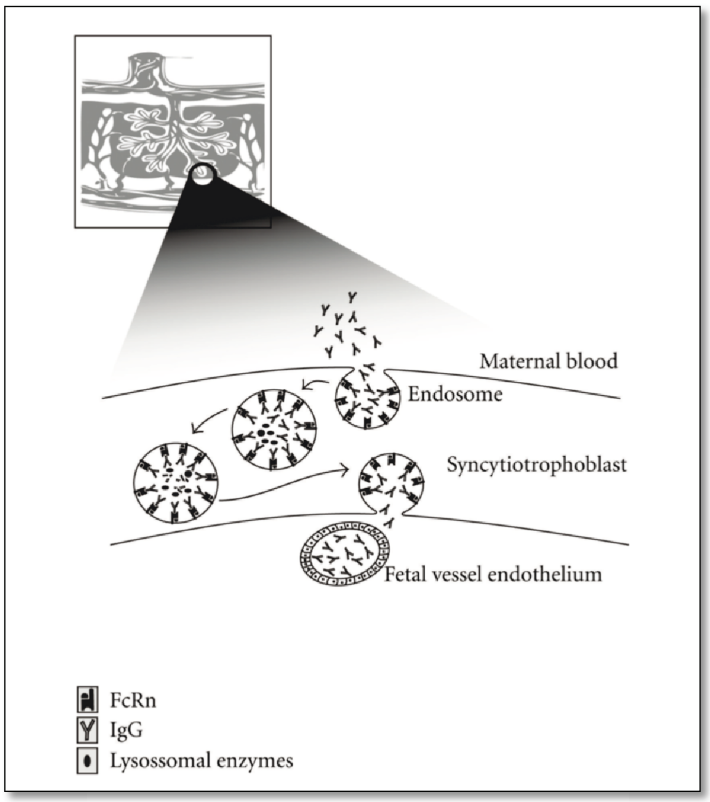
Among the five classes of immunoglobulin, only IgG, especially subclass IgG1, is known to transported transplacentally; IgG2, IgG3 and IgG4 are transferred to a lesser extent, with IgG2 in the lowest concentrations.[73] Differences in subclass transport are not completely understood, but it has been hypothesized that IgG1 is transported earlier than IgG2 or is somehow preferentially selected in the transport process.[73]

The majority of IgG is transported during the last four weeks of gestation.[74] Full term infants typically have a 20-30% greater concentration of IgG than their mothers at the time of birth, resulting in a cord to maternal titer ratio (CMTR) of >1.0.[74] Maternal weight, age, parity and type of delivery do not appear to influence transplacental transfer of antibody.[74]

1.3.2.1 Mechanism of Transport

While this process is not yet completely understood, significant progress in understanding transplacental transport of antibodies has been made in recent years as reviewed by Palmeira et al.[74] In order for molecules to pass from maternal blood to fetal blood, they must cross the histological barrier. This is comprised of two cell layers: the multinucleated syncytiotrophoblasts (STB) and the endothelial cells of the fetal capillaries.[74] Nutrients and solutes can be effectively transported across this barrier through active or passive means, but with the exception of IgG, larger molecules generally do not cross.[74] (See **Figure 1.2**, from Palmeira, et al[74]) Transport of IgG is mediated by FcRn, which has a high affinity for binding to IgG, although only under pH conditions that favor transport toward the fetal blood supply.[74] Binding to FcRn can also become saturated, indicating that the amount of transfer is also dependent on the quantity of available cell surface receptors.

Figure 1.2: Transplacental Antibody Transfer (Palmeira, et al)



When maternal IgG levels are very high (above 15 g/L), ratios of cord to maternal IgG may be lower than when the maternal IgG concentration is low.[74] Studies have also shown that low birth weight infants have reduced levels of IgG1 and IgG2; suggesting an association between low birth weight and impaired placental transfer of IgG.[74] Expression of FcRn is tied to gestational age, with the greatest expression occurring in the third trimester. Therefore, total IgG concentrations are also highly dependent on length of gestation.[74]

1.3.3 Factors that can impair transplacental transfer of antibodies: HIV, malaria in pregnancy and hypergammaglobulinemia

As previously discussed, gestational age, IgG subclass and maternal concentration of antibody during pregnancy are important in determining the concentration of antibody transferred to infants, while maternal age and weight, parity and method of delivery do not appear to be related.[74] Placental abnormalities, injury, and conditions such as hypergammaglobulinemia, HIV, and placental malaria may also impair transfer.[74]

1.3.3.1 HIV

A recent study conducted in South Africa found that among HIV positive women with exposed but uninfected infants, transplacental transfer of antibodies for *Haemophilus influenzae* type B (Hib), pertussis and tetanus were reduced, likely due to two factors: lower levels of maternal antibody during pregnancy and reduced efficiency of transplacental transfer.[75] These results were consistent with findings from two previous studies of HIV-infected women in Kenya that demonstrated decreased transfer for tetanus and measles.[76, 77] However, the HIV-exposed but uninfected infants mounted robust responses to routine immunizations, which in some cases exceeded the responses of HIV-unexposed infants. The authors hypothesize that this improved response resulted from decreased levels of maternal antibody, leading to decreased antibody-mediated suppression of the infant immune response, or less neutralization of vaccine virus replication, in the case of measles.[75] The impact of HIV on transplacental transfer of antibodies and infant response to vaccines needs to be studied further, both alone and in combination with *Plasmodium falciparum* and *Plasmodium vivax* malaria in pregnancy.

1.3.3.2 Malaria in Pregnancy

Despite comprehensive malaria control efforts, there are an estimated 214 million cases of malaria each year and 438,000 deaths, and more than 125 million women become pregnant each year in malaria endemic regions.[78, 79] A disproportionately high fraction of the malaria disease burden is borne by pregnant women and their fetuses. Significant risks are associated with malaria in pregnancy, and pregnant women are at greater risk for infection and complications of malaria than non-pregnant women, including severe anemia and death.[78]

Malaria in pregnancy can also cause inflammation and monocytic infiltration of the placenta as well as increased risk for preterm delivery, both of which can impair antibody transfer. *P. falciparum* infection of the placenta causes three specific changes: 1. Accumulation of infected erythrocytes in the intervillous space, 2. Infiltration of the intervillous space by monocytes and macrophages, some containing hemozoin, and 3. Hemozoin in fibrin deposits.[78] These changes result in inflammation, which then impairs transfer of nutrients and molecules from mother to fetus and can predispose for both intrauterine growth restriction (IUGR) and low birth weight (LBW) (<2500 grams) infants. Acute infection and high parasitemia have been associated with pre term birth, whereas chronic placental inflammation is associated with IUGR.[80] While the exact mechanisms of this process are not yet understood, cytokines and maternal and fetal insulin-like growth factors (which participate in the regulation of fetal growth) also appear to be disturbed.[81]

Several studies have found associations between placental malaria and impairment of transplacental transfer or antibodies, yet in most cases, those studies were unable to control for the effect of hypergammaglobulinemia or to evaluate the conditions separately, and often included pre-term infants.

1.3.3.3 Hypergammaglobulinemia

Hypergammaglobulinemia is an immunoproliferative disorder resulting in excessive production of IgG. It is believed to interfere with active receptor-mediated transfer of maternal antibody via saturation of placental FcRN receptors,[74] and impaired antibody transfer has been well-described in a number of maternal conditions that are associated with hypergammaglobulinemia, including HIV infection.[75, 77] Hypergammaglobulinemia has been associated with impaired transport of other virus-specific antibodies, including measles, [82-85] but further study of the role of hypergammaglobulinemia in transfer of RSV antibody independent of placental malaria and HIV is needed.

1.3.4 Potential threats to the effectiveness of maternal immunization for RSV

Studies of Ab transfer for measles, varicella, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and tetanus in the Gambia[71, 85], Malawi[83], and Kenya[76] have found associations between impaired transport and placental malaria, as did a study of tetanus Ab transfer among women with malaria in PNG[86]. Data on placental malaria and transplacental transport of RSV Ab are more limited; however, one study of 213 mother-infant pairs in the Gambia found that both placental malaria (defined by histology and blood smear of placental tissue) and hypergammaglobulinemia (defined as total IgG ≥1500 g/dL) were associated with a significant reduction in RSV IgG transfer as measured by ELISA. [71]

If HIV, placental malaria, and/or hypergammaglobulinemia are associated with impairment of transplacental transfer of RSV antibody, this may undermine maternal immunization programs for RSV in malaria endemic areas. Further study in this area is needed before maternal vaccines for RSV become available. [See Objective 1]

1.4 Project rationale: Challenges pertaining to clinical trials and implementation of RSV vaccines

As we proceed in the development of vaccines for RSV, a number of important questions remain to be answered. Prevention of disease through vaccination is the most promising way to reduce morbidity and mortality from RSV, particularly in low and middle-income settings where prophylaxis is cost-prohibitive and oxygen therapy is limited or unavailable. Maternal immunization is an attractive mechanism, as it could protect infants during the most vulnerable period of life, and could be particularly helpful in settings where antenatal care and immunization infrastructure is established, yet supportive care (oxygen therapy) for severe RSV is limited.

However, protection of the infant via maternal immunization requires sufficient transplacental transfer of antibodies across the placenta to the fetus. In many areas where the need for a maternal vaccine against RSV would be greatest, conditions such as HIV, malaria and hypergammaglobulinemia that have been shown to impair transfer of vaccine-derived antibodies either directly or indirectly are prevalent. There is an urgent need to better understand how these conditions could undermine maternal immunization for RSV in particular. There is a paucity of data on the transplacental transfer of RSV-specific antibody, and data on impairment of that transfer are further limited, particularly in non-African settings.

Furthermore, there is an underappreciation of both the burden and seasonality of RSV and its contribution either alone or via co-infection to LRI in low and middle-income settings in tropical equatorial regions. Seasonality studies in these areas have demonstrated a heterogeneity across and even within countries, highlighting the need for studies of viral LRI in a variety of representative settings. As the control of bacterial pneumonia improves with access to vaccines for pneumococcus, Hib, and pertussis, viral causes of pneumonia will become increasingly important. Modern molecular techniques can enable characterization of the seasonality and impact of respiratory viruses in such settings.

Thirdly, the spectrum of illness associated with RSV infection is broad. Because vaccine-induced sterilizing immunity is not expected (as re-infection throughout life is common), vaccine efficacy in clinical trials will need to be evaluated in terms of reduction of severe disease. To guide these efforts, data are needed to help evaluate case definitions for RSV disease of varying severity. Furthermore, clinical signs and symptoms that are associated with or predictive of severe disease need to be defined to help determine which infants are at greatest risk of complications.

1.5 Thesis objectives

The focus of this doctoral thesis was to investigate the following three objectives:

1.5.1 Objective 1

- Evaluate transplacental transfer of naturally-acquired RSV neutralizing antibody using paired maternal and cord blood specimens. RSV neutralizing antibody will be measured via plaque-reduction neutralization (PRN) assay

- Determine whether histologically-confirmed *Plasmodium falciparum* or *Plasmodium vivax* placental malaria is associated with impaired transplacental transport of RSV neutralizing antibody

- Determine whether maternal hypergammaglobulinemia is associated with impaired transplacental transport of RSV neutralizing antibody

    - These studies will be conducted in a rural and peri-urban coastal area of northern Papua New Guinea, a tropically equatorial country, using specimens from two temporally disparate cohorts of mothers and infants

1.5.2 Objective 2

Describe the epidemiology of pneumonia and circulating respiratory viruses in a cohort of infants followed from birth to age two in northern coastal Papua New Guinea, including description of co-infections, seasonality, and pathogen attributable fractions for severe and very severe pneumonia. Respiratory viruses will be detected via multiplex PCR of nasopharyngeal (NP) specimens collected during active and passive surveillance.

1.5.3 Objective 3

To inform upcoming phase III efficacy trials for RSV vaccines:

- Create objective case definitions for the differentiation of life-threatening and non-life threatening RSV LRI
- Analyze the predictive value of clinical signs and symptoms for development of those outcomes using data from hospitalized infants 0-2 years of age in Buenos Aires, Argentina.

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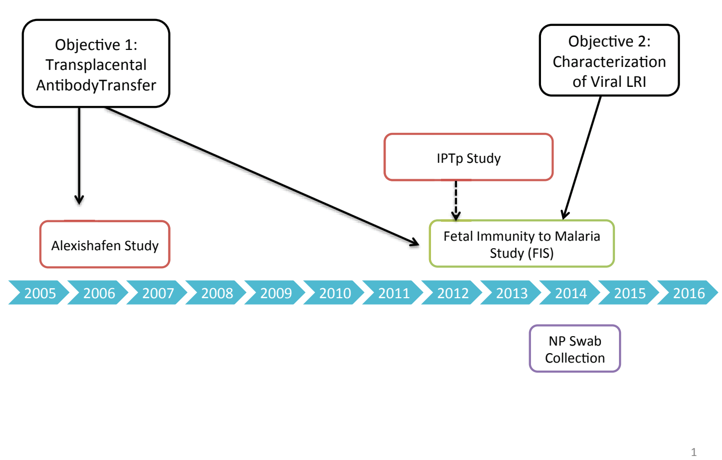
Chapter 2: Parent studies and field sites

Details pertaining to laboratory assays and statistical methods for each objective are contained in subsequent objective-specific chapters (chapters 3, 4 and 5). Each of these studies was nested within a parent study, and this chapter outlines additional information about those parent studies to provide context for the results to follow.

2.1 Sites and studies in Papua New Guinea

Objectives 1 and 2 were nested within larger projects conducted in rural and peri-urban areas on the north coast of Papua New Guinea by the Papua New Guinea Institute of Medical Research (PNGIMR) in collaboration with the University of Melbourne and Case Western Reserve University (CWRU) (**Figure 2.1**). We leveraged both data and specimens from existing studies of malaria in pregnancy and infancy for our RSV-specific aims.

Figure 2.1: Timeline of studies in Papua New Guinea



Objective 1 evaluates transplacental transfer of RSV antibodies in specimens from two cohorts of mothers and newborns. These studies were temporally distinct, but conducted in the same general area of Madang province. We refer to them here as the Alexishafen Study and the Fetal Immunity to Malaria (FIS) study. Objective 2 derives data from the FIS study only.

2.1.1 Alexishafen Study

The Alexishafen Study was designed to investigate risk factors for malaria in pregnancy and adverse birth outcomes.[1] Pregnant women were enrolled between September 2005 and October 2007 and followed until delivery at the Alexishafen Health Centre in Madang Province, on the north coast of Papua New Guinea, near Madang town. Women 16 years of age and older with no history of delivery complications and hemoglobin values ≥5 g/dL were eligible for the study. Prevalence of histologically confirmed placental malaria in this cohort was 60%.

For objective 1 of this thesis project, IRB and PNGIMR ethics approvals were granted to test maternal peripheral blood specimens collected on the day of delivery and cord blood specimens collected shortly after birth from the Alexishafen cohort for measurement of RSV neutralizing antibody. Baseline maternal data and infant outcome data were also available. Specimens had been stored at -80°C in Papua New Guinea and at CWRU since collection and were transported to Johns Hopkins for testing.

2.1.2 Fetal Immunity to Malaria Study (FIS)

The Fetal Immunity to Malaria (FIS) study was designed to evaluate the impact of malaria in pregnancy on the risk and severity of malaria from birth until age two, as well as a number of immunologic outcomes in infants. The FIS study was nested in a much larger clinical trial of intermittent preventive treatment of malaria in pregnancy (IPTp) evaluating sulphadoxine-pyrimethamine plus azithromycin versus sulplhadoxine-pyrimethamine and chloroquine for prevention of low birth weight and the prevalence of malaria and anemia at delivery.[2]

Approximately 340 of the 2,793 women participating the IPTp trial who gave birth between September 2011 and February 2014 consented for enrollment in FIS. FIS infants were followed from birth until age 2, with follow-up ending in February 2015. The prevalence of histologically confirmed placental malaria in this cohort was 9%.

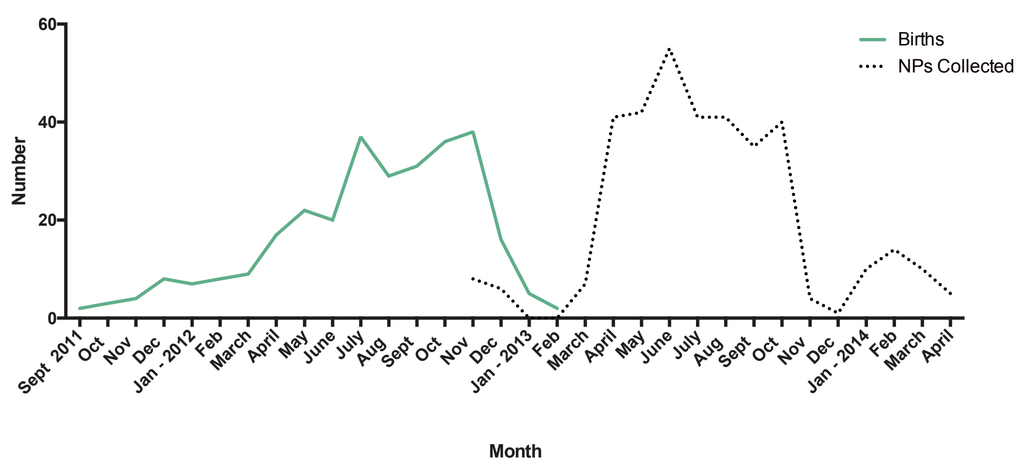
Infants in the FIS study were followed via active surveillance every 3 months (in rural areas) or 6 months (in peri-urban areas). Study participants also had access to local clinics where parents could seek care for acute illnesses. Nasopharyngeal (NP) swabs were collected from children presenting with any signs or symptoms of respiratory infection during scheduled surveillance visits (active surveillance) or during acute illness visits (passive surveillance) from November 2013 through February 2015.

For objective 1 of this thesis project, IRB and PNGIMR ethics approvals were granted to test maternal peripheral blood specimens collected on the day of delivery and cord blood specimens collected shortly after birth from the FIS cohort for measurement of RSV neutralizing antibody. For objective 2, NP specimens were collected on our behalf by study nurses and transported to Johns Hopkins for molecular testing. Baseline maternal data (objectives 1 and 2) and longitudinal infant outcome data (objective 2) were also available.

2.1.2.1 Limitations to specimen collection for objective 2

Figure 2.2 depicts the births of infants in the FIS study (solid green line) and the collection of NP swabs for objective 2 (dotted black line). Unfortunately, NP collection did not begin until November 2013, after the majority of births had occurred. Furthermore, because births occurred over a 17-month span, the age and number of children in follow-up at any given time point varied greatly. The limitations of these aspects of the study design will be discussed further in chapters 5 and 7.

Figure 2.2: FIS cohort births and NP swab collection



2.2 Sites and studies in Argentina

The analysis presented in objective 3 (chapter 5) was conducted using data from infants ≤24 months who were hospitalized with LRI in 2013 in and around Buenos Aires, Argentina. These data were provided by colleagues at Fundación INFANT, and represent a subset of children enrolled in a prospective study to assess the burden of RSV disease in a low-income population (PI Fernando Polack, Fundacion INFANT and Vanderbilt University; funding provided by BMGF). Approximately 56,000 infants in and around Buenos Aires served by 12 public hospitals were the source population for these studies.[3]

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Chapter 3: Impact of placental malaria and hypergammaglobulinemia on transplacental transfer of respiratory syncytial virus antibody in Papua New Guinea

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Jessica E. Atwell[1], Bhagvanji Thumar[1], Leanne J. Robinson[2,3,4], Roselyn Tobby[2], Phantica Yambo[2], Maria Ome-Kaius[2], Peter M. Siba[2,5], Holger W. Unger[6], Stephen J. Rogerson[6], Christopher L. King[7], Ruth A. Karron[1]

[1] Center for Immunization Research, Department of International Health Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Papua New Guinea Institute of Medical Research, Madang & Goroka, Papua New Guinea, [3]Walter and Eliza Hall Institute, Parkville, Victoria, Australia [4]Department of Medical Biology, the University of Melbourne, Australia, [5]College of Public Health, Medical and Veterinary Sciences Division of Tropical Health and Medicine, James Cook University, Australia, [6]Department of Medicine, The University of Melbourne, Melbourne, Australia, [7]Center for Global Health and Disease, Case Western Reserve University and Veterans Affairs Medical Center, Cleveland, OH, United States

3.1 Abstract

BACKGROUND:

Passively acquired respiratory syncytial virus (RSV)-neutralizing antibody protects against RSV-associated lower respiratory infections but placental malaria (PM) and maternal hypergammaglobulinemia might interfere with transplacental immunoglobulin transport.

METHODS:

We measured RSV plaque reduction neutralization (PRN) antibody titers in 300 full-term maternal/cord serum pairs in two cohorts in malaria-endemic Papua New Guinea: Alexishafen (2005-08) and FIS (2011-13). We defined impaired transport as a cord to maternal titer ratio (CMTR) <1.0 and a protective RSV PRN titer (PRNT) as ≥1:200.

RESULTS:

PM and hypergammaglobulinemia occurred in 60% and 54% of Alexishafen mothers versus 8% and 9% of FIS mothers, respectively. 34% of Alexishafen and 32% of FIS pairs demonstrated impaired transport. Multivariate modeling revealed significant associations between increasing maternal IgG ($\log_2$) and impaired transport (adjusted OR, Alexishafen: 2.68 [1.17-6.14], FIS: 6.94; [1.94-24.8]) but no association with PM. 34% of Alexishafen and 31% of FIS cord PRNTs were <1:200.

CONCLUSIONS:

Impaired RSV Ab transport was observed in ~one-third of maternal/cord pairs. Hypergammaglobulinemia, but not PM, was associated with impaired transport, particularly among women with low RSV PRNT. Detection of RSV PRNT <1:200 in one-third of cord sera supports efforts to increase levels of RSV neutralizing antibody in pregnant women through maternal immunization.

KEY WORDS: RSV, transplacental transfer of antibody, maternal immunization, placental malaria, hypergammaglobulinemia

3.2 Introduction

Pneumonia is the leading global cause of morbidity and mortality in children under 5 years of age.[1] As access to vaccines for bacterial pneumonia improves, viruses such as influenza and respiratory syncytial virus (RSV) are becoming increasingly important contributors to acute lower respiratory illness (ALRI) in pediatric populations. RSV is the most frequent cause of ALRI and bronchiolitis in infants.[2, 3] A recent global estimate of the annual burden attributed 33.8 million episodes of illness in children under 5 years of age and 253,000 deaths in children under 1 year to RSV; 99% of deaths are estimated to occur in developing countries.[1, 3]

Protection against RSV-associated disease is primarily antibody (Ab)-mediated[4, 5] and passively acquired RSV-neutralizing Ab can protect infants against RSV ALRI.[6-9] To address the global burden of RSV, promising vaccines are in development with a focus on maternal immunization to prevent ALRI in the first months of life and infant immunization to prevent ALRI in later infancy and early childhood.[10-12]

Protection of infants via maternal immunization requires efficient transplacental transport of maternal IgG.[13] Transport begins during the second trimester and increases until delivery, with peak transfer occurring after 32 weeks gestation.[13] In term infants, cord IgG typically equals or exceeds maternal IgG.[13] Preterm birth, maternal malaria (specifically, placental malaria [PM]), HIV, and hypergammaglobulinemia have been associated with impaired transplacental transfer of IgG for a variety of pathogens;[13-26] however, data are limited on transplacental transport of RSV Ab,[7, 20, 22, 27-29] as well as conditions that may impede transport of RSV-specific Ab.[20, 22] Therefore, we sought to determine the impact of PM and maternal hypergammaglobulinemia on transplacental transport of RSV-neutralizing Ab. Furthermore, we restricted our study to term infants in order to evaluate effects of PM independent of its association with preterm birth. We conducted our study using specimens from mother-infant pairs residing on the north coast of Papua New Guinea (PNG), where both *Plasmodium falciparum* and *Plasmodium vivax* malaria are prevalent and pneumonia is the leading cause of child mortality, accounting for 17% of deaths in children under 5.[30]

3.3 Methods

3.3.1 Populations and specimens

Our study was nested within two studies of malaria in pregnancy conducted by the Papua New Guinea Institute of Medical Research (PNGIMR), the University of Melbourne, and Case Western Reserve University. These studies were conducted in rural and peri-urban areas on the north coast of Madang Province, PNG (**Figure 3.1**). We analyzed 300 pairs of maternal and cord sera collected at delivery from term pregnancies (gestational age [GA] ≥37 weeks).

The first cohort, "Alexishafen", provided 157 maternal/cord pairs obtained between 2005-2008 near Madang town (**Figure 3.1**), prior to intensified malaria control interventions and when the rate of malaria transmission was very high.[31, 32] The second cohort, from the Fetal

Immunity Study ("FIS"), provided 143 pairs from a clinical trial of intermittent preventive treatment of malaria in pregnancy (IPTp) (NCT01136850).[33] Pregnant women from regions in or near Madang town were enrolled between 2011-2013. As this was subsequent to initiation of malaria control measures in 2009-10,[34] the rate of malaria in pregnancy was expected to be substantially lower than in the Alexishafen cohort.

GA at delivery was assessed using Ballard scores in the Alexishafen cohort,[35] and composite measures of GA based on last menstrual period, fundal height, Ballard scores, and ultrasound data, when available, in the FIS cohort.[33]

3.3.2 Placental histology

Histologic characterization of placentae was performed using methods developed by Rogerson et al.[33] Placentae were graded 1-5 to indicate acute (stages 1 and 2), chronic (3), past (4), or no (5) infection. We defined PM as the presence of parasites, malaria pigment, or both (stages 1-4). PCR and blood smears for malaria were also performed on maternal peripheral blood and placentae using methods described elsewhere for Alexishafen[32] and FIS.[33]

3.3.3 Maternal IgG Measurement

Maternal IgG levels were measured by radial immunodiffusion (Binding Site, Birmingham, UK). Maternal hypergammaglobulinemia was defined as ≥1700 mg/dL, based on comparisons to levels in healthy adults.[36]

3.3.4 RSV-specific antibody measurement

RSV Ab was measured using a complement-enhanced 60% plaque-reduction neutralization (PRN) assay.[37] Plaque-reduction neutralizing antibody titers (PRNT) are expressed arithmetically and as reciprocal $\log_2$.

Cord to maternal titer ratios (CMTRs) (cord PRNT/maternal PRNT) were calculated for each mother-infant pair. CMTRs in healthy, full-term pregnancies range from 1.0–1.2.[13] Therefore, for this study, we defined normal transfer as CMTR≥1.0, impaired transfer as CMTR<1.0, and severely impaired transfer as CMTR<0.8.

Cord PRNT was also assessed as an outcome of interest. A cord PRNT that correlates with protection of infants against RSV ALRI has yet to be precisely defined. To determine an appropriate threshold for our analysis, we reviewed data from studies in cotton rats and in infants of protection afforded by intravenous administration of immunoglobulin containing high titers of RSV neutralizing Ab (RSV-IGIV) measured using a similar PRN assay.[4, 9] In cotton rats, PRNTs of 1:200-1:400 were associated with protection against pulmonary infection.[4] In high-risk infants receiving monthly doses of RSV-IGIV, trough levels of serum RSV Ab as measured by PRNT were generally >1:200 in infants in whom protection against RSV ALRI was observed.[9] Therefore, we categorized infants with cord blood PRNT ≥1:200 as having the putative minimal Ab level required for protection against RSV ALRI at birth.

3.3.5 Statistical analysis

Statistical analyses were performed using Stata version 11 (StataCorp LP, College Station, Texas, USA). χ^2, Fisher's exact, Mann-Whitney, and Student's t-tests were used for pairwise comparisons as appropriate. Linear regression was used for continuous outcomes, while logistic regression was used for dichotomous outcomes. P <0.05 was considered significant. Log_2 transformed maternal RSV PRNT, categorical maternal age, and gravidity (dichotomized as primigravid and non-primigravid) were included in final models *a priori*. Total IgG was analyzed as both a continuous and dichotomized predictor.

3.3.6 Ethical review

The Institutional Review Boards of University Hospitals Case Medical Center (No. 05-11-02), the Papua New Guinea Medical Research Advisory Committee (No. 11.33), and the Johns Hopkins School of Public Health approved this work.

3.4 Results

3.4.1 Mothers and infants

In Alexishafen and FIS, the mean maternal age was 25 years; range, 16-49 (Alexishafen), and 16-42 years (FIS) (**Table 3.1**). Fewer women in Alexishafen were primigravid compared to FIS (34.4% vs. 48.9%, P=0.012). At delivery, maternal anemia (hemoglobin ≤9 g/dL) was substantially higher in Alexishafen than in FIS (40% vs. 17%; P<0.001, Table 1).

Approximately 7% of infants in each cohort were low birth weight (<2500 grams) (**Table 3.1**).

3.4.2 Malaria in pregnancy

The burden of malaria differed substantially between cohorts. In Alexishafen, 92% of mothers had ≥1 PCR+ assay for malaria in peripheral blood, 43% were malaria+ at delivery by blood smear, and 60% of placentae showed evidence of chronic or acute PM. In contrast, the frequency of malaria in FIS was substantially lower: 9% of pregnant women had ≥1 PCR+ assay for malaria in peripheral blood, 2.9% of mothers were malaria+ at delivery by blood smear, and 8.8% of placentae for which histology was available showed evidence of PM (**Table 3.1**).

3.4.3 CMTRs, placental malaria, and hypergammaglobulinemia

The distribution of CMTRs was very similar across the two cohorts: geometric mean (GM) 1.19 for Alexishafen (range, 0.22-4.54) and 1.22 (range, 0.29-3.88) for FIS. Despite the vast difference in rates of PM between the cohorts, proportions of maternal-cord pairs with impaired transfer were nearly identical. Approximately one third (34% in Alexishafen, 32% in FIS) of maternal-cord pairs in each cohort had CMTR <1.0 [**Table 3.2; Figure 3.2A and 4D**]. Furthermore, the proportions with impaired transfer were not statistically significantly different after stratification by PM exposure: 37% of PM-exposed pairs in Alexishafen and 25% in FIS had CMTRs<1.0 (**Table 3.2, Figure 3.2B and 4E**), compared to 32% and 34% respectively in PM-unexposed pairs (**Table 3.2, Figure 3.2C and 4F**). Severely impaired transfer (CMTR <0.8) was observed in 17% of Alexishafen and 18% of

FIS pairs and likewise was not significantly different after stratification by PM exposure (**Table 3.2**). The relationship between PM and CMTR was further explored through univariate and multivariate logistic regression, adjusting for maternal PRNT, maternal IgG, maternal age, and gravidity (**Table 3.3**). These analyses confirmed that PM was not significantly associated with impaired transfer in either cohort. Regression analyses using PCR-based definitions of malaria exposure (any positive PCR test in antenatal care (ANC) or at delivery) were also conducted; no significant associations were observed (data not shown).

Maternal IgG levels were much greater in Alexishafen [GM: 2004 (range, 727–7514 mg/dL)] than FIS [GM: 1110 (range, 501-2762 mg/dL), $P<0.001$] as was the proportion of women with hypergammaglobulinemia (54% vs. 8%, $P<0.001$; **Figure 3.3**). Interestingly, PM and hypergammaglobulinemia were not invariably associated: of 300 total women studied, 105 had PM and 97 had hypergammaglobulinemia, but only 53 had both (**Figure 3.4**).

In contrast to PM, high levels of maternal IgG were associated with impaired transfer of RSV Ab in these populations. We found that mean CMTR were slightly lower when maternal hypergammaglobulinemia was present than when it was absent (GM CMTR 1.07; 95% CI 0.96-1.19 vs. 1.26; 95% CI 1.19-1.34; $P=0.05$)(**Figure 3.5**). However, multivariate analyses that included IgG and CMTR as continuous variables and adjusted for maternal PRNT, maternal age, gravidity, and PM demonstrated that increasing maternal IgG was significantly associated with a reduction in CMTR in both cohorts. Likewise, increasing maternal IgG was associated with increased odds of impaired CMTR when we dichotomized at CMTRs of 1.0 and 0.8. For every doubling of maternal IgG, the odds of a CMTR<1.0

increased substantially in both cohorts (adjusted OR for CMTR<1.0 for Alexishafen: 2.68; 95% CI 1.17-6.14, P=0.02; FIS: 6.94; 95% CI 1.94-24.8, P=0.003; **Table 3.3**). Hypergammaglobulinemia was not invariably associated with high maternal levels of RSV PRNT: 36% of women with IgG levels >1700 had RSV PRNT <1:200 (**Figure 3.6**). Furthermore, the effect of hypergammaglobulinemia on transplacental transport appeared to be greatest in those with the lowest titers: among women with both hypergammaglobulinemia and maternal RSV PRNT <1:200, 39% had CMTR <1.0, whereas only 21% of women with RSV PRNT <1:200 and normal IgG levels had CMTR <1.0 (P=0.04).

3.4.4 Titers of RSV neutralizing antibodies in maternal and cord sera

Maternal RSV PRNT GMT were nearly identical in both cohorts (Alexishafen: GMT 254, range 11-7150; FIS: GMT 239, range 19-2259), as were cord RSV PRNT GMTs (Alexishafen: GMT 302, range 21-3257; FIS: GMT 291, range 23-2592). The point estimates for mean maternal PRNT were lower in women with PM than in those without PM in Alexishafen and FIS, but these findings were not statistically significant (**Table 3.2**). As expected, maternal and cord PRNT were highly correlated (Pearson's r: 0.82 (P<0.001) in Alexishafen, 0.73 (P<0.001) in FIS, **Figure 3.2**).

34% of cord sera from Alexishafen and 31% from FIS had RSV PRNT <1:200 (**Figure 3.7**). While not evaluated in these cohorts, studies conducted elsewhere estimate the half life of maternally-derived RSV Ab to be ~28 days.[27, 28] Using this decay rate, the proportion of 1-month-old infants with a cord PRNT <1:200 would increase to 66% and 68% in Alexishafen and FIS, respectively. Interestingly, the infant specimens with cord PRNT <1:200 and the maternal/infant paired specimens with a CMTR <1.0 were somewhat distinct: only 55% of Alexishafen infants and 41% of FIS infants met both criteria (**Figure 3.4b**).

3.5 Discussion

As maternal immunization becomes an increasingly important strategy for protection of very young infants, identification of factors that could impact vaccine effectiveness by modifying the efficiency of transplacental Ab transport will be critical. This study shows that increasing maternal IgG, but not PM, was associated with impaired transport of RSV-neutralizing Ab in mothers and full term infants in PNG, and that impaired transport occurred in approximately one-third of maternal-cord pairs.

These cohorts provided an unusual opportunity to examine associations between maternal conditions and impaired Ab transport. Participants were from catchment areas in close proximity to one another but were enrolled in 2005-2008 (Alexishafen) or in 2011-2013 (FIS). During this time interval, regional intensification of malaria control led to substantial reduction in rates of malaria[32] yet the pattern of transplacental transport of RSV neutralizing Ab remained nearly identical, with no statistically significant associations between PM and impaired transport demonstrated in either univariate or multivariate models.

Our findings differ from previous reports of the effect of PM on transplacental Ab transfer. Studies of Ab transfer for measles, varicella, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and tetanus in the Gambia,[20, 21] Malawi,[17] and Kenya[15] have found associations between impaired transport and PM, as did a study of tetanus Ab transfer among women with malaria in PNG.[14] Data on PM and transplacental transport of RSV Ab are more limited; however, one study of 213 mother-infant pairs in the Gambia[20] found that both PM (defined by histology and blood smear of placental tissue) and hypergammaglobulinemia (defined as total IgG ≥1500 g/dL) were associated with a significant reduction in RSV IgG transfer as measured by ELISA.

There are several possible explanations for the differences between these earlier findings and ours. First, we restricted our study to term infants to assess the effect of PM independent of preterm birth. In contrast, the study in The Gambia[20] only excluded infants born at <24 weeks gestation, and therefore 27% of infants studied were pre-term. PM is a known cause of preterm birth, and preterm birth is associated with impaired Ab transfer.[13] We wanted to evaluate associations between PM and impaired transfer independent of preterm birth. Second, we measured RSV-neutralizing Ab (rather than total RSV IgG) because it correlates with protection against ALRI.[6-9] It is possible that the IgG subclass distribution found when measuring RSV-neutralizing Ab is different from the distribution found when measuring total RSV IgG (which presumably would contain both neutralizing and non-neutralizing Ab), and that PM might have a differential effect on subclass transport. Finally, the study in The Gambia evaluated the effects of both PM and hypergammaglobulinemia, but 94% of the hypergammaglobulinemic women also had PM, complicating the assessment of those exposures independently. In contrast, only 56% of the hypergammaglobulinemic women in our study had PM, allowing us to more readily examine the separate effects of these two conditions (**Figure 3.4a**). Our evaluation of maternal IgG as a continuous predictor may have also helped us retain important detail in these associations, especially in the FIS cohort, where few women had IgG values >1700 mg/dL. Of interest, we found that mean RSV PRNT was lower in mothers with PM compared to those without PM, though these differences were not significant (**Table 3.2**). This finding should be explored further in future studies.

We showed that increasing maternal IgG was significantly associated with impaired transport of RSV neutralizing Ab independent of maternal age, gravidity, PM, and maternal RSV PRNT, and that its impact may be more pronounced among women with lower RSV PRNT. Hypergammaglobulinemia is believed to interfere with active receptor-mediated transfer of maternal Ab via saturation of placental FcRN receptors,[13] and impaired Ab transfer has been well-described in a number of maternal conditions that are associated with hypergammaglobulinemia, including HIV infection.[19, 23] Hypergammaglobulinemia has been associated with impaired transport of other virus-specific Abs, including measles.[16, 17, 21, 38] Interestingly, women in Alexishafen and FIS had strikingly different rates of hypergammaglobulinemia (54% vs. 8%) despite being separated by only a few years in time. While some general health indicators have improved in this interval,[30] the substantial decrease in malaria prevalence in this area of PNG is likely the greatest change and may partially explain this difference. Thus, while malaria does not appear to impair Ab transfer directly (i.e. through infection of the placenta) it may impair transfer indirectly through induction of hypergammaglobulinemia. Further studies into the causes of hypergammaglobulinemia in this region are needed.

Despite the association between hypergammaglobulinemia and impaired transport, cord PRN GMT were strikingly similar in the Alexishafen and FIS cohorts. This is likely because maternal RSV PRNT remains the most important predictor of cord RSV PRNT on a population basis. In both cohorts, we found that approximately one half of mothers and one third of infants had RSV PRNTs below the putative protective threshold of 1:200. Taking into account the decay of maternal antibodies after birth, the proportion of susceptible infants would increase to 66%-68% by 1 month of age. These data suggest that a large proportion of young infants in PNG may be susceptible to severe RSV infection. Additional studies to determine the epidemiology of RSV in this population are underway.

This work was subject to several limitations. Specimens were obtained from studies designed for other purposes, so our ability to evaluate additional exposures related to hypergammaglobulinemia is limited. HIV status was not available for either cohort, but a study among women seeking ANC in Madang during 2011-13 found HIV prevalence to be 1.1%.[33] Syphilis serostatus was not available for women in Alexishafen, but only 2 women in FIS had serologic evidence of active syphilis infection at enrollment. Preterm birth was an exclusion criterion for this study. For a large proportion of participants, methods other than ultrasound were used to estimate GA. Imprecise GA assessment could result in failure to exclude preterm infants, however, recent evidence suggests that these clinical methods have a high specificity for preterm birth.[39] Finally, while PRN is considered the gold standard for measurement of RSV Ab, it is a bioassay and subject to variability. We attempted to minimize this by using standardized protocols and reagents and by testing maternal and cord sera in duplicate and on the same plate to ensure consistency.

In summary, we have evaluated transplacental transfer of RSV neutralizing Ab in northern coastal PNG. We showed that increasing maternal IgG, but not PM, was associated with impaired transport among full term infants. Moreover, we showed that approximately one-third of full-term infants were born without protective levels of RSV neutralizing Ab. These data suggest that approaches to enhance passive protection against RSV in very young infants in populations such as this might help prevent ALRI in early life. As maternal immunization strategies against RSV and other pathogens are developed and implemented, it will be important to continue to assess factors that affect transplacental transfer of Ab and to determine whether sufficient levels of Ab can be induced to overcome any potential deficits in transport.

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3.7 Tables and Figures

Table 3.1: Baseline Clinical Characteristics of Mother Infant Pairs

	Alexishafen Cohort	FIS Cohort	*P* value
Number of pairs	157	143	
Maternal Characteristics			
Maternal age in years, mean (range)	25.4 (16-49)	25 (16-42)	*0.543*
Gravidity, mean (range)	2.77 (1-10)	1.8 (1-8)	*<0.001*
Primigravid, n (%)	54 (34.4%)	70 (48.9%)	*0.012*
Maternal hemoglobin at delivery in g/dL, mean (range)	9.3 (4.2 – 14.2)	9.67 (5.5 – 13.9)	*0.097*
Anemic, defined as ≤9 g/dL,n (%)	63 (40%)	25 (17%)	*<0.001*
Infant Characteristics			
Gestational age at birth in days, mean (range)	273 (245-294)	277 (255-300) days	*<0.001*
Birth weight in grams, mean (range)	3007 (2050 – 5700)	3057 (2100-4400)	*0.324*
Low birth weight, defined as <2500g, n (%)	11 (7%)	10 (6.9%)	*0.996*
Female, n (%)	70 (46.4%)	69 (48.3%)	*0.7451*
Placental Malaria by Histology			
Number of pairs with histology available	153	137	
Placental malaria positive (stage 1-4), n (%)	93 (60.2%)	12 (8.8%)	*<0.001*
Placental malaria negative (stage 5) n (%)	60 (39.2%)	125 (91.2%)	*<0.001*
Stage 2 – Acute: parasites, pigment in monocytes +/- fibrin	20 (13%)	0	*<0.001*
Stage 3 – Chronic: parasites, pigment in fibrin	3 (2.0%)	1 (0.7%)	*0.361*
Stage 4 – past infection	12 (7.8%)	4 (2.9%)	*0.062*
Peripheral Blood PCR			
No positive PCR test in pregnancy	13 (8.2%)	130 (90.9%)	*<0.001*
Any positive PCR test in pregnancy (*P. falciparum, P. vivax, or P. ovale*)	144 (91.7%)	13 (9.1%)	*<0.001*
Negative by PCR at delivery (maternal peripheral blood)	90 (57.3%)	136 (97.1%)	*<0.001*
Positive by PCR at delivery (maternal peripheral blood)	67 (42.7%)	4 (2.9%)	*<0.001*
Maternal IgG			
Mean (range)	2004 (727.8 – 7514.3)	1110 (501 – 2726.2)	*<0.001*
Hypergammaglobulinemia (>1700 mh/dL) n (%)	85 (54%)	12 (8.4%)	*<0.001*

Table 3.2 RSV PRN Titers and cord to maternal titer ratios (CMTR) by placental malaria and hypergammaglobulinemia status

	Maternal PRNT GMT (range)	Cord PRNT GMT (range)	CMTR GM (range)	Proportion of CMTRs <0.8	Proportion of CMTRs <1.0
Alexishafen Cohort					
All	254.3 (11-7150)	301.7 (21.4 – 3257)	1.19 (0.22-4.54)	0.17	0.34
Placental Malaria +	220.4 (23-7150)	256.0 (33 – 3257)	1.16 (0.22 – 4.24)	0.17	0.37
Placental Malaria -	303.0 (11-3329)	371.7 (21.4 – 3191)	1.23 (0.32 – 4.54)	0.17	0.32
	P=0.02	*P*=0.007	*P*=0.8	OR 1.04 [0.40 – 2.77] *P*=0.9	OR 1.24 [0.59 – 2.64] *P*=0.53
Hypergammaglobulinemia +	305.1 (11-7150)	332.4 (21.4 – 3257)	1.09 (0.22 – 4.55)	0.22	0.42
Hypergammaglobulinemia -	205.1 (23-1973)	269.1 (33-2870)	1.31 (0.40 – 3.99)	0.11	0.25
	P=0.004	*P*=0.159	*P*=0.014	OR 2.3 [0.88-6.5; *P*=0.06]	OR 2.2 [1.05 – 4.66, *P*=0.02]
FIS Cohort					
All	239.1 (19.5 – 2259)	291.0 (23.1-2592)	1.22 (0.29 – 3.88)	0.18	0.32
Placental Malaria +	229.9 (58.7 – 587.9)	300.9 (78.3 – 869.3)	1.30 (0.71 – 2.55)	0.08	0.25
Placental Malaria -	246.0 (19.5 – 2259)	295.2 (23.1 -2592.8)	1.2 (0.29 – 3.88)	0.2	0.34
	P=0.81	*P*=0.78	*P*=0.52	OR 0.36 [0.008 – 2.74] *P*=0.46	OR 0.64 [0.11 -2.73] *P*=0.75
Hypergammaglobulinemia +	368.5 (87.1 – 2032)	353.2 (192.4 - 836)	0.96 (0.41 – 2.58)	0.50	0.67
Hypergammaglobulinemia -	229.8 (19.5 – 2259)	285.9 (23.1 – 2598)	1.24 (0.29 – 3.88)	0.15	0.29
	P=0.055	*P*=0.278	*P*=0.065	OR 5.55 [1.31 -22.7] *P*=0.009	OR 4.89 [1.2 -23.3] *P*=0.019

Table 3.3: Multiple Logistic Regression for association between placental malaria and maternal IgG with Cord to Maternal Titer Ratios (CMTRs)

	Alexishafen		FIS	
	Adjusted Odds Ratio (CI)	*P* value	Adjusted Odds Ratio (CI)	*P* value
CMTR <0.8				
Placental Malaria	1.11 (0.43-2.87)	0.822	0.237 (0.024-2.27)	0.212
Maternal IgG	2.93 (1.10-7.74)	0.03	5.95 (1.32-26.77)	0.02
CMTR <1.0				
Placental Malaria	1.36 (0.647-2.87)	0.415	0.44 (0.099-1.94)	0.280
Maternal IgG	2.68 (1.17-6.14)	0.020	6.94 (1.94-24.8)	0.003

Placental malaria is included in these models as a dichotomous variable, while maternal IgG (measured in mg/dL) is a $\log_2$ transformed continuous measure.

Figure 3.1 Papua New Guinea

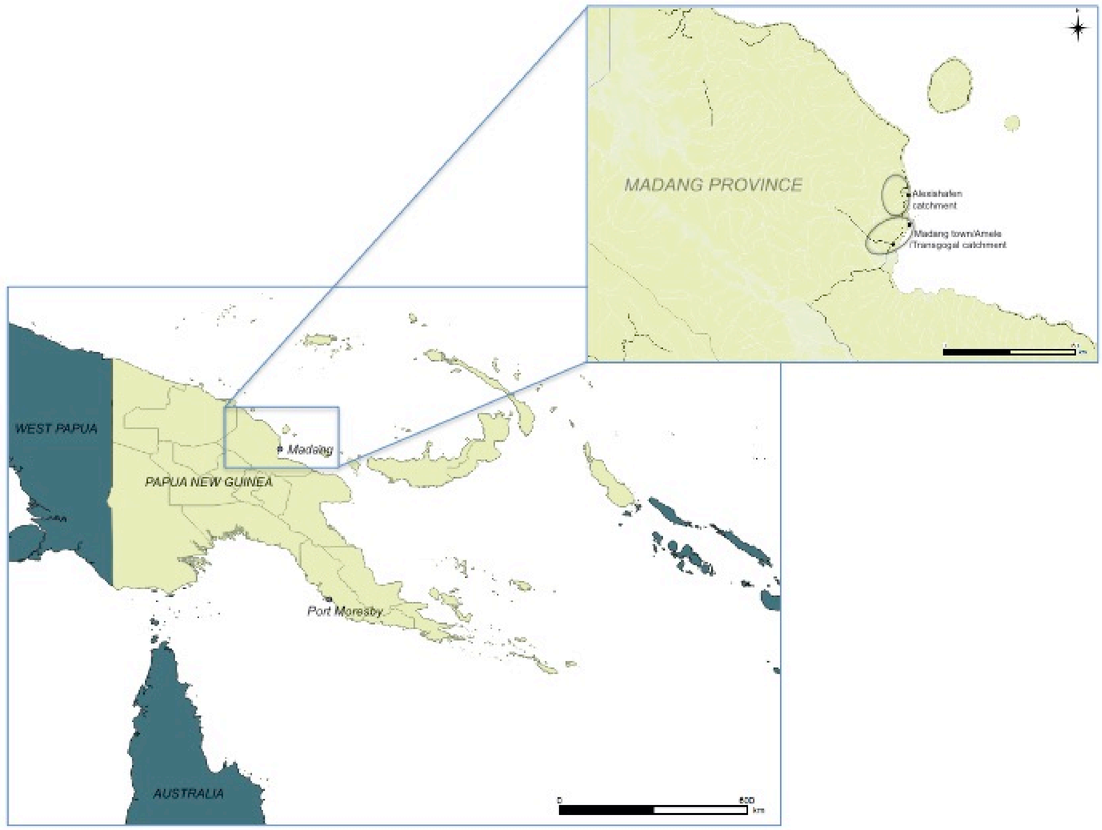

Figure 3.1 Map of Papua New Guinea. Madang Province is located on the northern coast, as shown on the map and inset.

Figure 3.2: Cord to maternal titer ratios

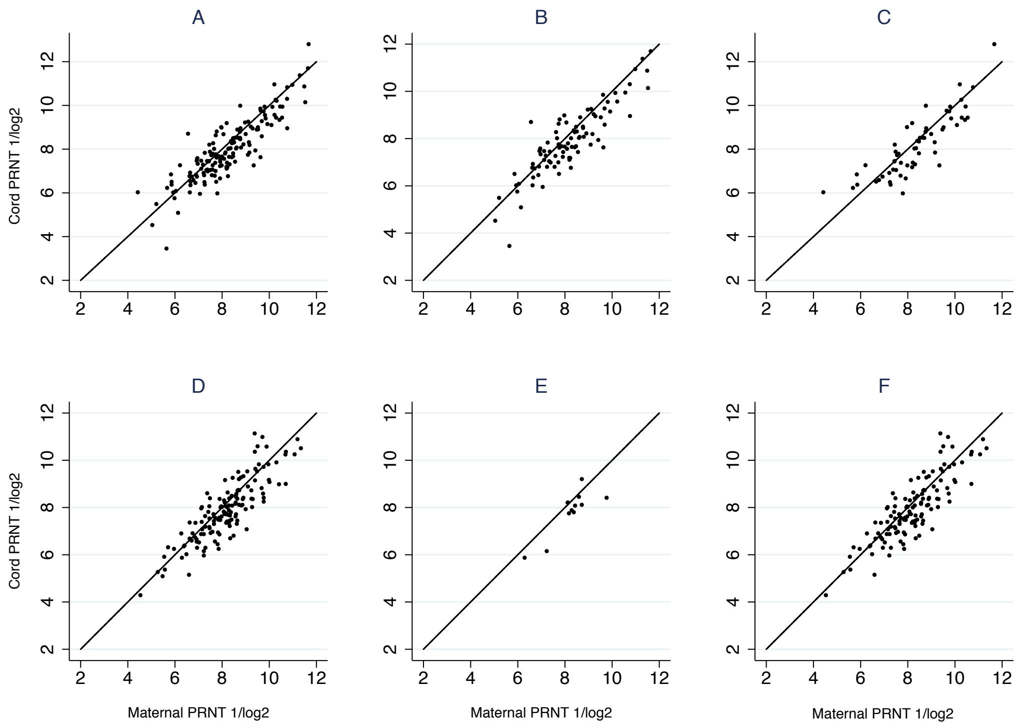

Figure 3.2: Relationship of maternal respiratory syncytial virus (RSV) plaque reduction neutralizing titer (PRNT) to cord RSV PRNT in the presence and absence of placental malaria (PM). PRNT are expressed as reciprocal $\log_2$. In each graph, the solid line represents a cord to maternal titer ratio (CMTR) of 1.0. A) Alexishafen cohort, all pairs; B) Alexishafen cohort, PM positive pairs; C) Alexishafen cohort, PM negative pairs; D) FIS cohort, all pairs; E) FIS cohort, PM positive pairs; F) FIS cohort, PM negative pairs.

Figure 3.3: Maternal IgG

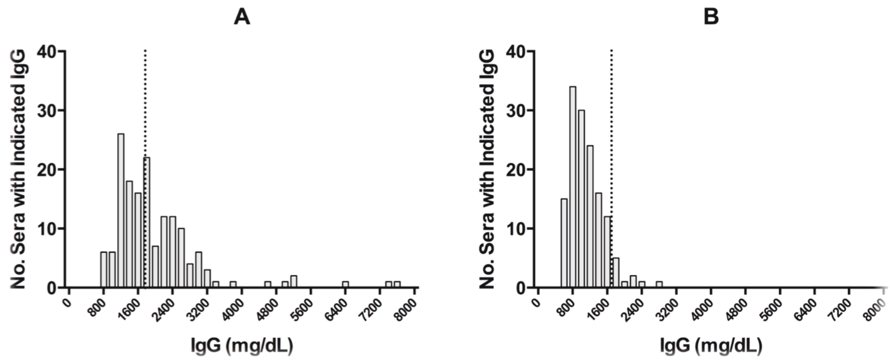

Figure 3.3: Distributions of maternal IgG in each study cohort. A) Alexishafen cohort, B) FIS cohort. IgG levels are expressed as mg/dL. The dashed line represents the threshold for hypergammaglobulinemia (1700 mg/dL).

Figure 3.4: Venn Diagrams of CMTR, cord titer, placental malaria and hypergammaglobulinemia

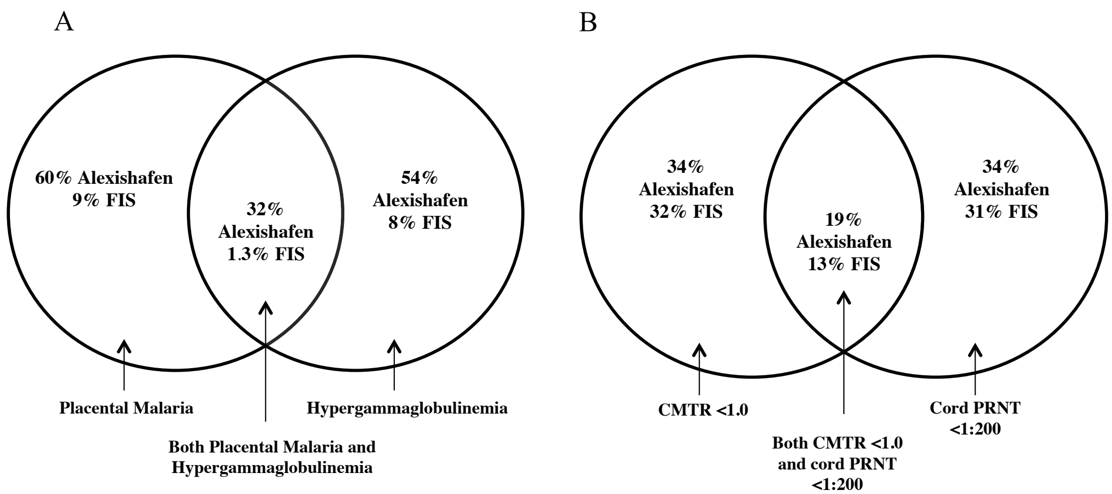

Figure 3.4: A) The relationship between placental malaria (PM) and hypergammaglobulinemia. Left circle, proportion of mothers with PM by histology; right circle, proportion of mothers with hypergammaglobulinemia, shaded middle area, proportion of mother with both PM and hypergammaglobulinemia. B) The relationship between cord to maternal titer ratios (CMTR) and cord plaque reduction neutralization titers (PRNT). Left circle, proportion of maternal/cord pairs exhibiting CMTR <1.0; right circle, proportion of cord specimens with PRNT <1:200, shaded middle area, proportion of cord specimens where CMTR was <1.0 and PRNT was < 1:200.

Figure 3.5: CMTR by hypergammaglobulinemia status

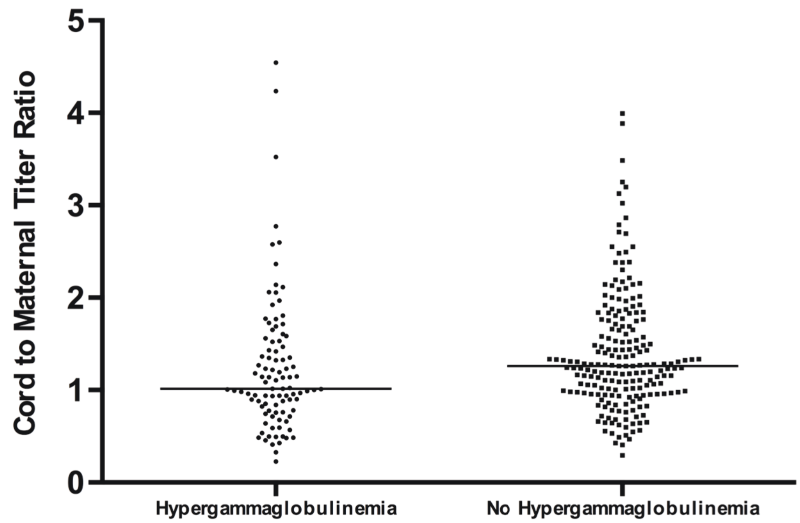

Figure 3.5: Distribution of cord to maternal titer ratios (CMTRs) by presence or absence of hypergammaglobulinemia. The Alexishafen and FIS cohorts are combined. The solid line represents the geometric mean CMTR for each group.

Figure 3.6 Maternal total IgG and RSV PRNT

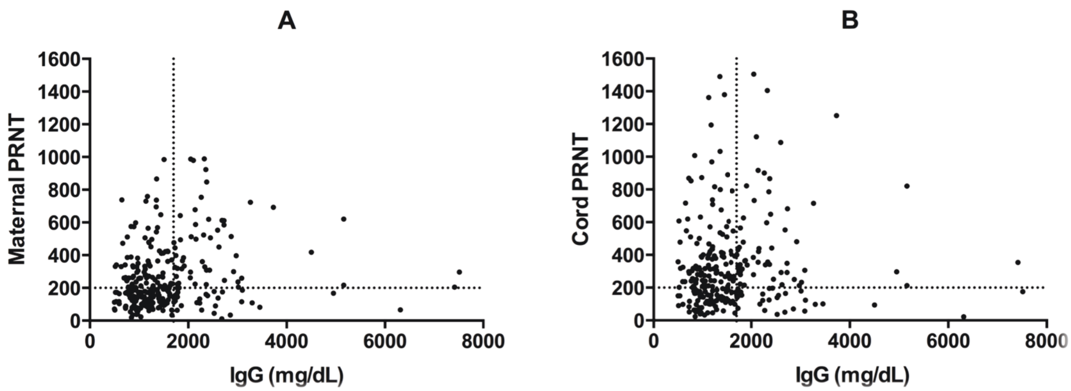

Figure 3.6: Maternal total IgG versus maternal RSV PRNT (A) and Cord RSV PRNT (B) for Alexishafen and FIS cohorts combined. Reference lines included to indicate hypergammglobulinemia (maternal total IgG of 1700 mg/dL) and the putative threshold of protective RSV PRNT (1:200). Note: two data points have been excluded from figure 3b for clarity. Total IgG levels for these points in the normal range (1) cord PRNT: 1:1729, total IgG: 1261 mg/dL (Alexishafen), (2) cord PRNT: 1:1663, total IgG: 760 mg/dL (FIS).

Figure 3.7: Reverse cumulative distribution of RSV PRNT by cohort

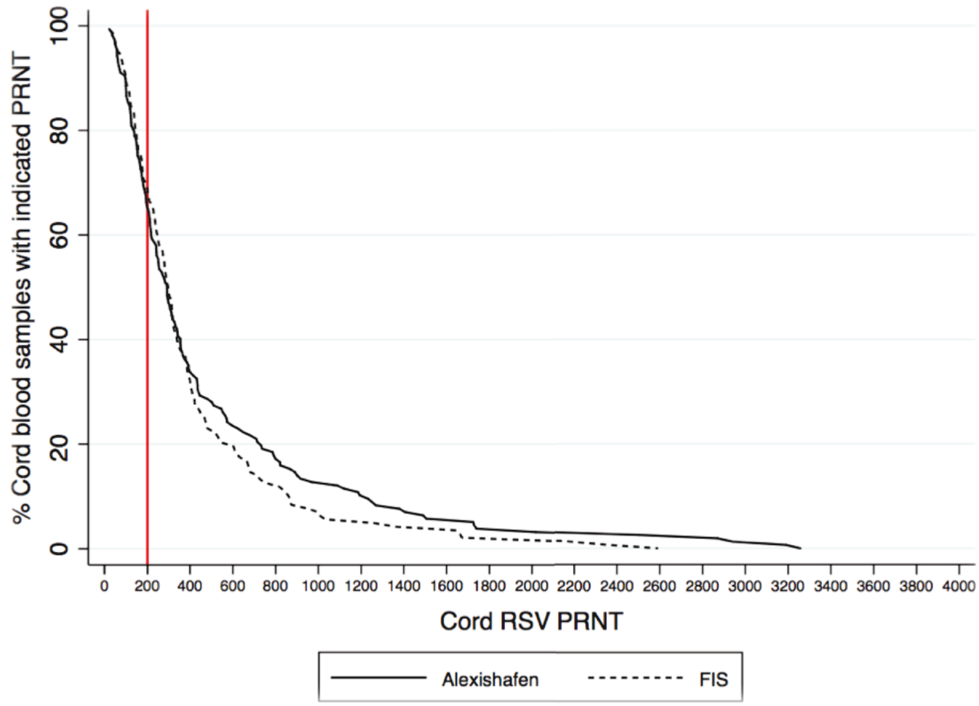

Figure 3.7: Reverse cumulative distribution of cord respiratory syncytial virus (RSV) plaque reduction neutralization titers (PRNTs) by cohort. The solid line represents the Alexishafen cohort and the dotted line represents the FIS cohort. The vertical solid line indicates a cord PRNT of 1:200.

Chapter 4: Etiology of viral pneumonia in children under two in coastal Papua New Guinea

Jessica E. Atwell[1], Bhagvanji Thumar[1], Jennifer Oliva[1], Roselyn Tobby[2], Maria Ome-Kaius[2], Leanne J. Robinson[2,3,4], Christopher L. King[5], Ruth A. Karron[1]

[1] Center for Immunization Research, Department of International Health Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Papua New Guinea Institute of Medical Research, Madang & Goroka, Papua New Guinea, [3]Walter and Eliza Hall Institute, Parkville, Victoria, Australia [4]Department of Medical Biology, the University of Melbourne, Australia, [5]Center for Global Health and Disease, Case Western Reserve University and Veterans Affairs Medical Center, Cleveland, OH, United States

In preparation

4.1 Abstract

Background:

Limited data are available on the seasonality and etiology of viral respiratory disease in Papua New Guinea (PNG), yet lower respiratory infections (LRI) are common causes of morbidity and pneumonia is the leading cause of death in children under five years of age. Seasonality of viruses such as RSV can vary greatly in tropical settings, necessitating country-specific data to inform prevention and treatment of viral LRI as well as prioritization and use of vaccines for RSV, which may soon be available.

Methods:

To assess the contributions of respiratory viruses including RSV to LRI in a cohort of infants in coastal PNG, we tested 360 nasopharyngeal (NP) swab specimens collected from

November 2012 – April 2014 using the Fast Track Diagnostics respiratory pathogens 21 assay. We evaluated seasonal distribution, co-infections, and pathogen attributable fractions (PAF) for severe and very severe pneumonia.

Results

Respiratory viruses were detected in 88% of nasal swabs. The most commonly detected viruses were rhinovirus (42%), bocavirus (28%), adenovirus (23%), and RSV A/B (11%). Co-infections, particularly pairwise combinations of rhinovirus, adenovirus and bocavirus were common. RSV was most commonly detected in April, May and June; no other viruses demonstrated strong seasonal patterns. Non-severe, severe and very severe pneumonia occurred year round, without variation by season. The PAF of RSV for severe pneumonia was 56% using non-severe pneumonia as the reference. Rhinovirus, adenovirus, and bocavirus were not associated with severe pneumonia.

Conclusions

Despite limited specimen availability from infants <5 months and overall detection of RSV in only 11% of nasal swab specimens, we identified a large PAF for RSV in cases of severe pneumonia. Furthermore, RSV was consistently detected in cases of severe pneumonia throughout the first two years of life, indicating its importance in older infants and children in this population. These data may help inform the treatment and prevention of respiratory infections in PNG as well as future active and passive immunization for RSV.

4.2 Introduction

Pneumonia is the leading cause of death among children under five globally. [1] Furthermore, in malaria-endemic areas, recent studies have suggested there may be a high rate of misdiagnosis of febrile illness as malaria coupled with an underappreciation of pneumonia, both bacterial and viral.[2, 3] In settings where vaccines for bacterial causes of pneumonia are becoming increasingly available, the proportion of pneumonia cases in children under five that can be attributed to respiratory viruses will likely increase. [4] Many viruses have been associated with lower respiratory infections (LRI), but respiratory syncytial virus (RSV) is the leading cause of acute viral LRI worldwide.[5, 6]

Several studies have been conducted to determine the seasonality and etiology of viral LRI in tropical settings,[7-12] but important gaps in understanding remain. As reviewed by Bloom-Feshbach et al,[7] temperate climates tend to consistently experience RSV LRI in winter months, but in tropical regions, there is greater heterogeneity of seasonality. Outbreaks occur concurrently with the rainy season in some settings, but exhibit different patterns elsewhere, perhaps influenced by humidity and temperature.[7] Seasonal fluctuations in mean maternal RSV antibody titers may also be related to seasonality of disease.[13] Ultimately, multiple factors are likely influencing the seasonal distribution of RSV and other respiratory viruses,[14-17] necessitating studies in many settings to inform prevention strategies. Furthermore, advancements in molecular diagnostics have made comprehensive testing for multiple agents feasible.[18]

With the exception of one study in the Highlands[10] there remains a paucity of published contemporary data on viral respiratory infections in Papua New Guinea (PNG) where the burden of LRI in children under five is significant. [19, 20] PNG is an extremely diverse country geographically, climactically and culturally. There is heterogeneity of disease burden, particularly between the cool, mountainous Highland regions and the tropical coastal areas, which have higher average temperature and humidity and are at much lower elevation.

We conducted this study to better understand the seasonality and etiologies of viral LRI in this coastal region of PNG. Since respiratory viruses are likely important contributors to LRI in this region, this information could help inform treatment and prevention of LRI and prioritization and use of RSV vaccines once such vaccines are available.

4.3 Methods

4.3.1 Study group and baseline data collected

This work was nested within a fetal immunity to malaria study (FIS) conducted by the Papua New Guinea Institute of Medical Research (PNGIMR) and Case Western Reserve University in and around Madang Province, on the north coast of PNG.

318 women were enrolled during pregnancy and followed prospectively through delivery. Their infants were then followed via active and passive surveillance from birth until age two. Active follow-up occurred either every 3 months (rural children) or every 6 months (children living in and around Madang town). Study participants also had access to five local

clinics where they could seek care for acute illness outside of normal follow-up visits. Births occurred between September 2011 and February 2013.

4.3.2 Specimen collection, transport and storage

Nasopharyngeal (NP) swabs were obtained from all children presenting with respiratory illnesses during either active or passive surveillance visits between November 2012 and April 2014. Clinical information on signs and symptoms, including respiratory rate, temperature, and presence and type of indrawing were also collected at each visit.

NP swab specimens were placed in PrimeStore medium (Longhorn Vaccines & Diagnostics, San Antonio, TX) or universal transport medium (Remel, Lenexa, KS) and stored in coolers with icepacks immediately after collection and for no more than five hours before being transported to the local hospital for storage at -80°C.

4.3.3 Detection of respiratory viruses

We performed rRT-PCR using the Fast-Track Diagnostics Respiratory Pathogens 21 Assay (Fast-Track Diagnostics Ltd, Sliema, Malta) for the detection of the following viruses: influenza A, influenza A (H1N1), influenza B, rhinovirus, coronaviruses NL63, 229E, OC43 and HKU1, parainfluenza 1, 2, 3, and 4, human metapneumovirus A/B, bocavirus, respiratory syncytial virus A/B, adenovirus, enterovirus, and parechovirus.[18]

Because of the inherent difficulties in attributing causation to specific pathogens in pneumonia studies, we calculated a pathogen attributable fraction (PAF) for the most commonly detected viruses by calculating the odds of being positive for each virus among children with severe or very severe pneumonia versus the odds of being positive for each virus among children with non-severe pneumonia, then used the equation: $PAF = OR - (1/OR)$. We chose to use children with non-severe pneumonia as the comparator rather than children with upper respiratory illness (URI) because very few nasal swabs were collected from children with URI (see Figure 4.5).

For the purposes of this study, we used published PERCH case definitions for pneumonia.[21] Very severe pneumonia was defined as cough or difficulty breathing plus one of the following danger signs: difficulty breastfeeding, difficulty drinking, vomiting, convulsions, lethargy, or unconsciousness. Severe pneumonia was defined as cough or difficulty breathing and lower chest wall indrawing (LCWI) but none of the danger signs listed above. Non-severe pneumonia was defined as cough or difficulty breathing, but none of the danger signs, and no LCWI. (See appendix 2)

4.3.4 Evaluation of maternal RSV titer in relation to seasonal detection of RSV in infants

Maternal peripheral blood from the day of delivery was available for 325 women from the parent IPTp study[22] within which the FIS cohort is nested. These women delivered across an 18-month period, from May 2010 through December 2012. RSV neutralizing antibody was measured in these specimens using a microneutralization assay with an ELISA readout[23] (manuscript in preparation, Atwell and Karron) and those data were used to evaluate the relationship between mean maternal RSV PRN titer (PRNT) and RSV detection in infants by month.

4.3.5 Ethical Review

The Papua New Guinea Medical Research Advisory Committee (No. 11.33), and the Institutional Review Boards of University Hospitals Case Medical Center (No. 05-11-02), and the Johns Hopkins School of Public Health approved this work.

4.3.6 Statistical analysis

Statistical analyses were conducted with Stata 14 (College Station, Texas). Student's t-tests, χ^2, and Fisher's exact tests were used for evaluation of differences in means and proportions, respectively.

4.4 Results

4.4.1 Viral detection

360 NP swabs were collected from 184 unique children of the 318 in follow-up. The mean number of swabs collected per child was 1.13, with a range of 0 to 7 (**Figure 4.1, panel A**) Among those with at least one swab collected, the mean number per child was 1.95. Mean age at collection was 11.1 months, with a range of 1-30 months. (**Figure 4.1, panel B**)

At least one virus was detected in 88% of NP specimens. Co-infections were common and the proportion positive for more than one virus was generally consistent across age strata. (**Figure 4.1 panel C**). Overall, 176 (49%) of specimens were positive for only one virus, 115 (32%) were positive for two viruses, 21 (6%) were positive for three viruses, and only 3 (<1%) were positive for four viruses.

The most commonly detected viruses were rhinovirus (42%), bocavirus (28%), adenovirus (23%), RSV A/B (11%), and influenza B (6%). All other viruses were detected in fewer than 4% of specimens (**Figure 4.2, panel A**). As with overall viral detection, within the 176 specimens that were positive for only one virus, the most commonly detected viruses were rhinovirus (43%), bocavirus (12.5%), adenovirus (12%) and RSV (10%) (**Figure 4.2 panel B**).

When two or more viruses were present, the most common combinations were rhinovirus and bocavirus (36 instances), rhinovirus and adenovirus (29 instances), and adenovirus and bocavirus (23 instances). RSV and bocavirus were present together in 23 specimens. All pairwise co-infections are summarized in **Table 4.1**.

Among specimens with three viruses present, rhinovirus, adenovirus and bocavirus was the most common combination (4 instances), followed by two instances each of rhinovirus, bocavirus, and RSV; rhinovirus, bocavirus and enterovirus; and adenovirus, bocavirus and RSV.

Figure 4.3 shows the proportion of swabs positive for each virus in each of several age groups, as well as those collected from children under in the first year of life, second year of life, and overall. In each case, the most prevalent viruses are highlighted in the legend to the right of the pie, and the RSV portion is offset. None of the swabs collected from children in the first three months of life were positive for RSV; however, very few specimens were collected in this age group (n=7). Rhinovirus, bocavirus, adenovirus and RSV were the most commonly detected viruses across all age groups. Influenza A was slightly more prevalent in the second year of life, but absolute numbers were small. Large differences in detection across age groups were not observed.

4.4.2 Viral Detection and Pneumonia

Signs and symptoms collected during morbidity visits for each of the 360 tested swabs were evaluated based upon the PERCH case definitions for non-severe, severe and very severe pneumonia.[21] Children were classified into four possible categories: very severe pneumonia, severe pneumonia, non-severe pneumonia and no pneumonia. Using these definitions, 4% of the specimens were collected from children with no pneumonia, 70% from non-severe pneumonia, 14% from severe pneumonia, and 12% from very severe pneumonia. **Figure 4.4** shows the distribution of total viruses detected (no viruses, one, two, three or four) as well as the total number of cases meeting each definition above the bar. There were no statistically significant relationships observed between severity of pneumonia and number of viruses detected.

Rhinovirus, bocavirus, and adenovirus were the most commonly detected viruses in all categories of pneumonia, as they were across all tested specimens. RSV was most prevalent in the severe pneumonia group. **Figure 4.5** shows the distribution of viruses detected, stratified by pneumonia category.

Using children with non-severe pneumonia as our reference category, there was an increase in the odds of RSV positivity in cases of severe pneumonia [OR 2.65; 95% CI (1.08 – 6.13), p:0.012], but not in cases of very severe pneumonia [OR 0.47; 95% CI (0.49 – 1.91), NS]. The PAF indicates that a substantial proportion of severe pneumonia in this population may be attributable to RSV (56%) (**Table 4.2**). In contrast, the OR for rhinovirus in cases of severe pneumonia was 0.66 (*P=NS)*, such that a PAF could not be calculated (**Table 4.2**). No other viruses were found to be associated with severe or very severe pneumonia **(Table 4.3).**

4.4.3 Seasonal Distribution of specific viruses

NP specimen collection began in November of 2012 and continued through April of 2014, although births of children in this cohort began in September of 2011. **Figure 4.6** shows NP collection in relation to births over calendar time. **Figure 4.7** shows detection of the most common viruses by month as a proportion of swabs tested (left axis), as the number of swabs collected each month was variable (dotted line, right axis). Most viruses including rhinovirus, bocavirus, and adenovirus were detected year-round, without strong seasonal patterns; however, the majority of the RSV cases (dotted red line) were detected in April, May and June. Sharp peaks are observed for H1N1 and influenza A in November to December of 2012 and November to January of 2014 respectively, but very few specimens were collected during those holiday months.

4.4.4 Seasonal Distribution of pneumonia

Non-severe, severe and very-severe pneumonia did not appear to vary by season; disease of all severity levels was present year round. **Figure 4.8** displays pneumonia over time as a proportion of the number of swabs tested by month (dotted line, right axis).

4.4.5 Cumulative incidence of pneumonia

2,036 active and passive visits between age 0 and 24 months occurred among the 318 children in follow-up. 873 of those visits among 292 unique children met a definition of non-severe, severe or very severe pneumonia, resulting in an estimated cumulative incidence of 2.8 cases of pneumonia per child. All three case definitions of pneumonia were more frequent among children age 0-12 months than among children 13-24 months. (**Table 4.4)**

4.4.6 Mean maternal RSV PRNT and detection of RSV in infants

Figure 4.9 shows the variation in mean maternal RSV PRNT titer by month of delivery among 325 mothers from the parent IPTp study plotted against RSV detection by month among infants in this cohort. RSV cases peaked in April, May and June. Mean maternal PRNT was lowest in the months directly preceding this peak (March, April, May), and highest in July following the peak in RSV detection, although this difference was not statistically significant.

4.5 Discussion

This cohort presented a unique opportunity to evaluate the presence of important respiratory viruses in a population with a significant burden of pneumonia, where studies of this kind had not previously been conducted. Advancements in both transport media and molecular diagnostics also enabled testing of specimens collected from a challenging environment where storage and transport of NP may be non-ideal according to historical standards, and where local molecular diagnostic capabilities are limited, due to the difficulty of importing necessary reagents and maintaining complex equipment.

Despite these limitations, respiratory viruses were frequently detected in these specimens; 88% of nasal swabs were positive for at least one virus, regardless of whether they were collected during passive or active surveillance. Consistent with other studies, rhinovirus, bocavirus, adenovirus, and RSV A/B were the most prevalent and co-infections with these viruses were common. LRI was observed year round.

This study design was not optimized to measure true burden of pneumonia, however morbidities meeting a PERCH definition of non-severe, severe or very severe pneumonia were frequent. Our estimate of cumulative incidence of 2.8 episodes per child is likely an underestimate, given that not all children were in follow-up for the full two years, and many cases of LRI may have been missed.

Our analysis was hindered by the paucity of data for the youngest infants. Only 7 swabs were collected from infants in the first three months of life, and none were positive for RSV. Unfortunately, NP collection did not begin until after the majority of births had occurred, meaning most infants were already beyond that age group. It is likely that we missed the greatest burden of RSV in this population. Despite this, the PAF of RSV for severe pneumonia was 56%. This contrasts sharply with rhinovirus, adenovirus and bocavirus, which were the most prevalent viruses, yet were not associated with severe pneumonia. Furthermore, RSV-associated severe pneumonia cases occurred across a wide range of ages (5 to 19 months, mean 11 months). While our data are insufficient to comment on the burden of RSV disease in the youngest infants, our findings indicate that RSV contributes substantially to severe pneumonia in older infants in this setting. These data suggest that

that this population may benefit from active RSV immunization of older infants as well as passive (maternal) immunization to combat the full burden of RSV.

The lack of attribution of RSV in cases of very severe pneumonia is consistent with other studies and may be related both to the lack of specificity of the WHO-derived case definition (which frequently identifies children with all-cause severe disease, not just pneumonia) as well as the likelihood that RSV infection may be less frequently associated with very severe pneumonia. We did not test for bacterial pathogens, but severe pneumonia cases may be more likely to be bacterial.

In addition to the limited data available for the youngest infants, this study was subject to a number of additional limitations. Very few specimens were collected from children who did not meet a case definition for pneumonia, and we were therefore unable to use URI controls as a reference group for our PAF calculations. Doing so would have been particularly helpful in contextualizing the importance of viruses commonly associated with URI (rhinovirus, bocavirus, adenovirus) but with uncertain relationships to LRI. In addition, using non-severe pneumonia as the referent may have led to calculation of a higher PAF for RSV as a cause of severe pneumonia.

This study was not optimized to accurately evaluate seasonality. The children in follow-up were born over a 17-month period and followed until age two, yet the delays in initiating NP collection restricted our ability to acquire specimens from children under one year of age across more than one calendar year. While we cannot make strong conclusions about seasonality in this cohort without multiple years of data, these findings are in agreement with published[10] and unpublished data from the region (P. Rarau, personal communication). Studies designed explicitly to determine both burden and seasonality in PNG should be conducted.

RSV neutralizing antibody levels from the 325 women in the parent IPTp were lowest among women delivering in March, April and May, the months directly preceding when peak RSV was observed among infants in this cohort. Maternal antibody was highest directly after peak detection of RSV (June and July). Other studies have observed similar patterns and have suggested that the seasonality of RSV in young infants may be driven in part by low maternal antibody titer and a loss of herd immunity among adults or that a cyclical relationship between disease and antibody levels in the population may exist.[13, 24, 25] Our findings suggest this phenomenon may be occurring in this setting as well. While it is difficult to know whether disease is driving maternal antibody levels, if maternal antibody levels are driving disease, or if both are true, increasing maternal antibody titers may help reduce illness in the youngest infants via passive protection of transplacental acquired antibody and herd immunity among adults. It is important to note that the maternal titers were pooled by month and were collected in different calendar years (2010-2012) than when the NP swabs were collected (2012-2014).

Furthermore, our previous work demonstrated that one third of infants in this same population were born with RSV PRNT below the threshold believed to be protective.[26] Those findings, coupled with the high detection rate of RSV across the first two years of life and the high PAF for severe pneumonia observed in this study provide further evidence that both passive and active immunization against RSV in this setting may be beneficial.

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4.7 Tables and Figures

Table 4.1: Co-infections

	Flu A	H1N1	Flu B	Rhino	Cor 43	Cor 63	Cor 229	Cor HKU	Para 2	Para 3	Para 4	Para 1	HMPV AB	HboV	RSV A/B	Adeno	EV	PV
Flu A				1						1			1	1		2		
H1N1				1										2				
Flu B				3							1			7		5		
Rhino	1	1	3		2	2				5	2		1	36	7	29	4	1
Cor 43				2										4	1	5		
Cor 63				2				1					1	3		3		
Cor 229																		
Cor HKU						1									1	1		
Para 2																		
Para 3	1			5										4	1	2		
Para 4			1	2											1	1		
Para 1																		
HMPB AB	1			1		1								2		2		
HboV	1	2	7	36	4	3				4			2		15	23	2	1
RSV A/B				7	1			1		1	1			15		4		1
Adeno	2		5	29	5	3		1		2	1		2	23	4		2	1
EV				4										2		2		
PV				1										1	1	1		

Table 4.2: Population Attributable Fractions

Pneumonia Severity	Negative	Positive	Total	Odds Ratio	CI	P-value	PAF [OR – (1/OR)]
RSV							
No pneumonia	13	1	14	0.70	(0.015 - 5.08)	NS	
Non-severe	229	25	254	ref	-	-	
Severe	38	11	49	**2.65**	**(1.08 - 6.13)**	**0.012**	**56%**
Very Severe	41	2	43	0.47	(0.49 - 1.91)	NS	
Rhinovirus							
No Pneumonia	6	8	14	1.82	0.53 – 6.54	NS	
Non-Severe	147	107	254	ref	-	-	-
Severe	33	16	49	0.66	0.32 – 1.31	NS	-
Very Severe	23	20	43	1.18	0.58 – 2.4	NS	
Bocavirus							
No Pneumonia	10	4	14	1.07	(0.23 – 3.88)	NS	
Non-Severe	185	69	254	ref	-	-	
Severe	33	16	49	1.29	(0.63 – 2.61)	NS	
Very Severe	31	12	43	1.04	(0.46 – 2.22)	NS	
Adenovirus							
No Pneumonia	11	3	14	0.92	(0.16 – 3.65)	NS	
Non-Severe	196	58	254	ref	-	-	
Severe	37	12	49	1.10	(0.49 – 2.23)	NS	
Very Severe	32	11	43	1.16	(0.50 – 2.55)	NS	

Table 4.3: Respiratory viruses and pneumonia

Virus detected	No pneumonia n (%)	Non-severe pneumonia n (%)	Severe pneumonia n (%)	Very severe pneumonia n (%)	P-value (Fisher's Exact)
Adenovirus	3 (21)	58 (23)	12 (25)	11 (26)	
Bocavirus	4 (29)	69 (27)	16 (33)	12 (28)	
Coronavirus 229E	-	-	-	-	
Coronavirus OC43	-	11 (4)	1 (2)	2 (5)	
Coronavirus NL63	-	7 (3)	1 (2)	1 (2)	
Coronavirus HKU1	-	4 (2)	-	-	
Entervorus	-	8 (3)	-	-	
Human metapneumovirus A/B	-	4 (2)	1 (2)	0	
Influenza A (non H1N1)	-	5 (2)	2 (4)	0	
Influenza A (H1N1)	-	4 (2)	1 (2)	0	
Influenza B	2 (14)	16 (6)	1 (2)	1 (2)	
Parainfluenza 1	-	2 (1)	-	-	
Parainfluenza 2	-	1 (0.4)	1 (2)	0	
Parainfluenza 3	-	11 (4)	1 (2)	4 (9)	
Parainfluenza 4	-	10 (4)	-	1 (2)	
Parechovirus (PV)	2 (14)	1 (0.4)	-	-	**0.012**
Rhinovirus	8 (57)	107 (42)	16 (33)	20 (47)	
RSV A/B	1 (7)	25 (10)	11 (23)	2 (5)	**0.044**

Table 4.4: Cumulative incidence of pneumonia

	0 – 12 months n, (%)	13 – 24 months n (%)	Total episodes among children 0-24 months
Non-severe pneumonia	362 (56%)	285 (44%)	647
Severe pneumonia	63 (62%)	39 (38%)	102
Very severe pneumonia	78 (63%)	46 (37%)	124
Total – any pneumonia	503 (58%)	370 (42%)	873

Figure 4.1: Summarizing NP collection

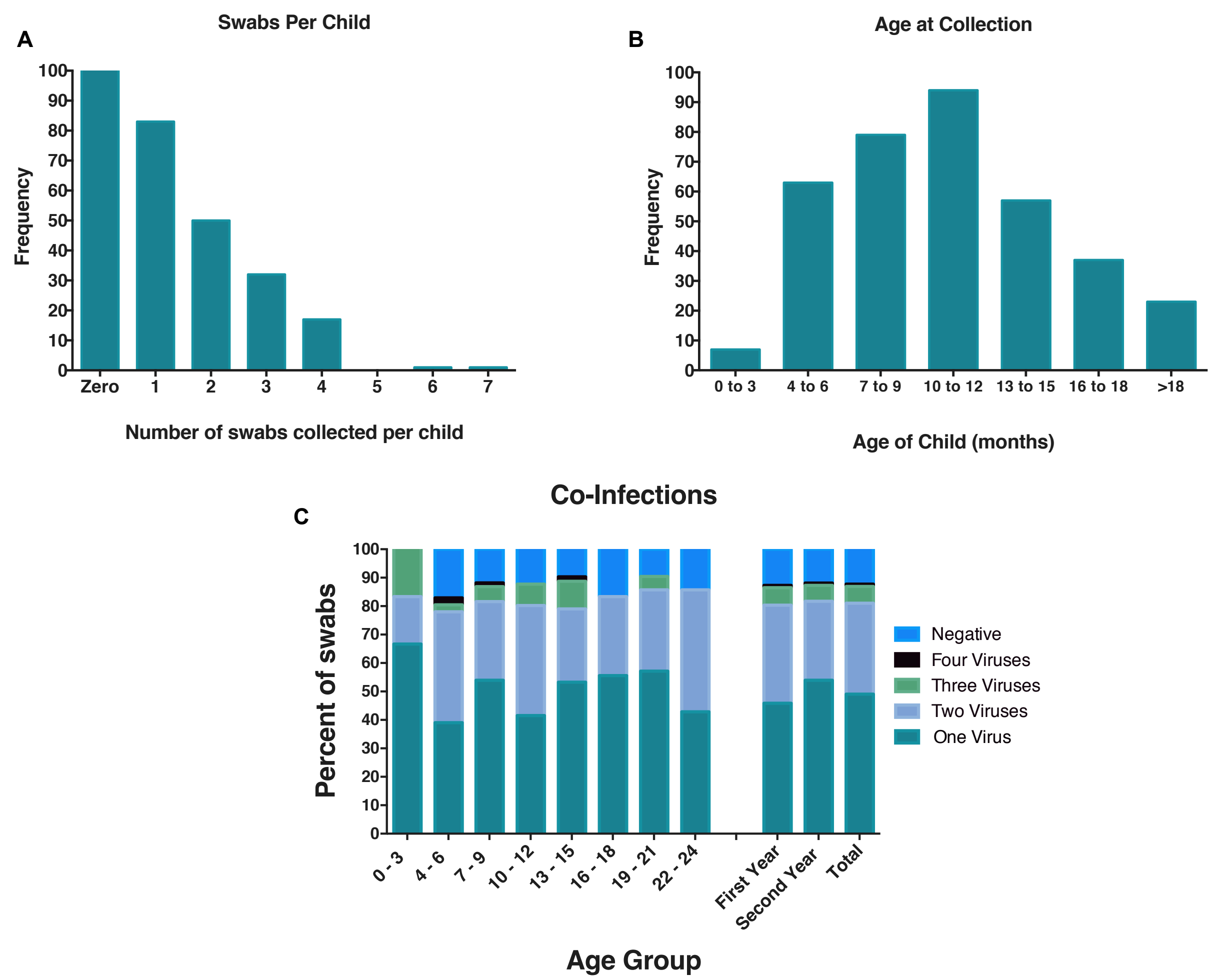


Figure 4.2: Overall virus detection

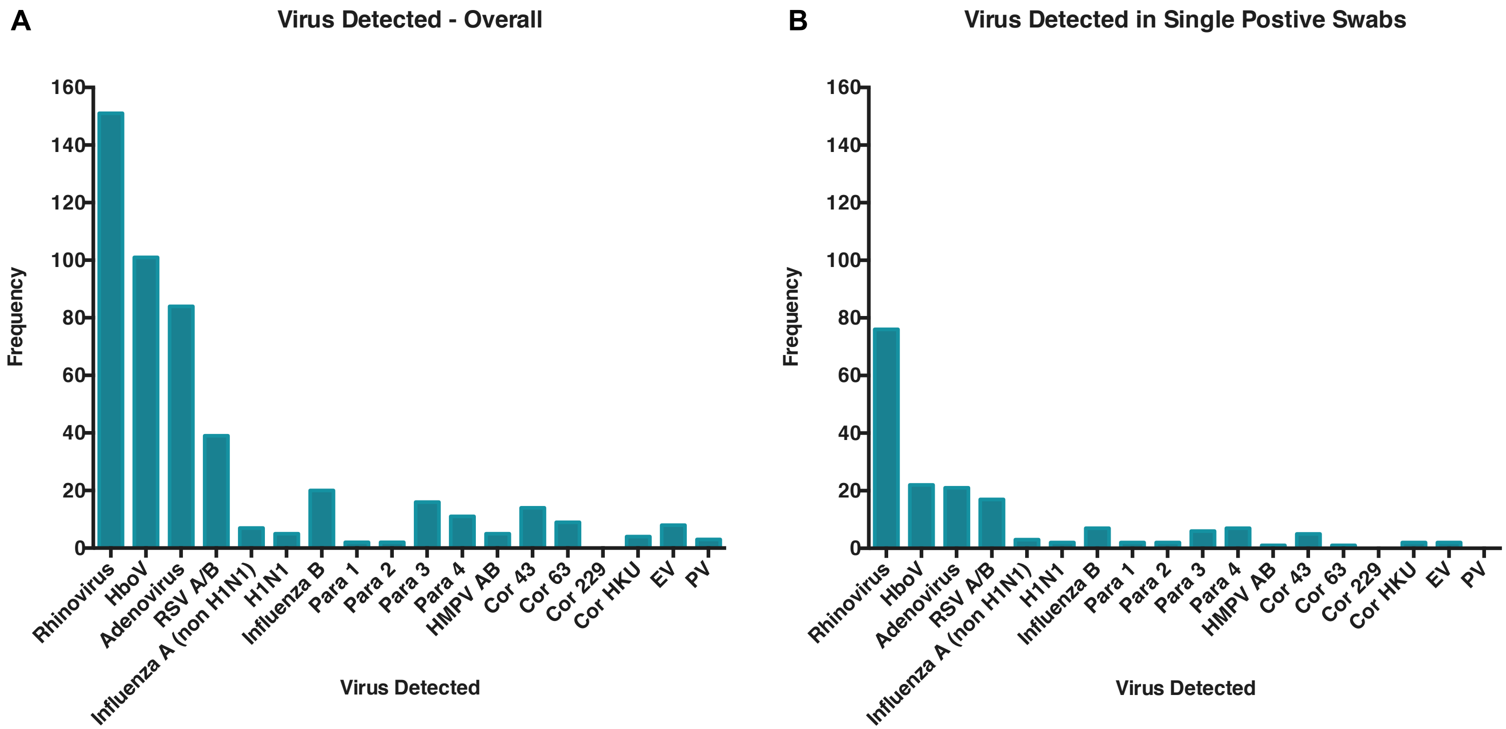


Figure 4.3: Viral detection by age

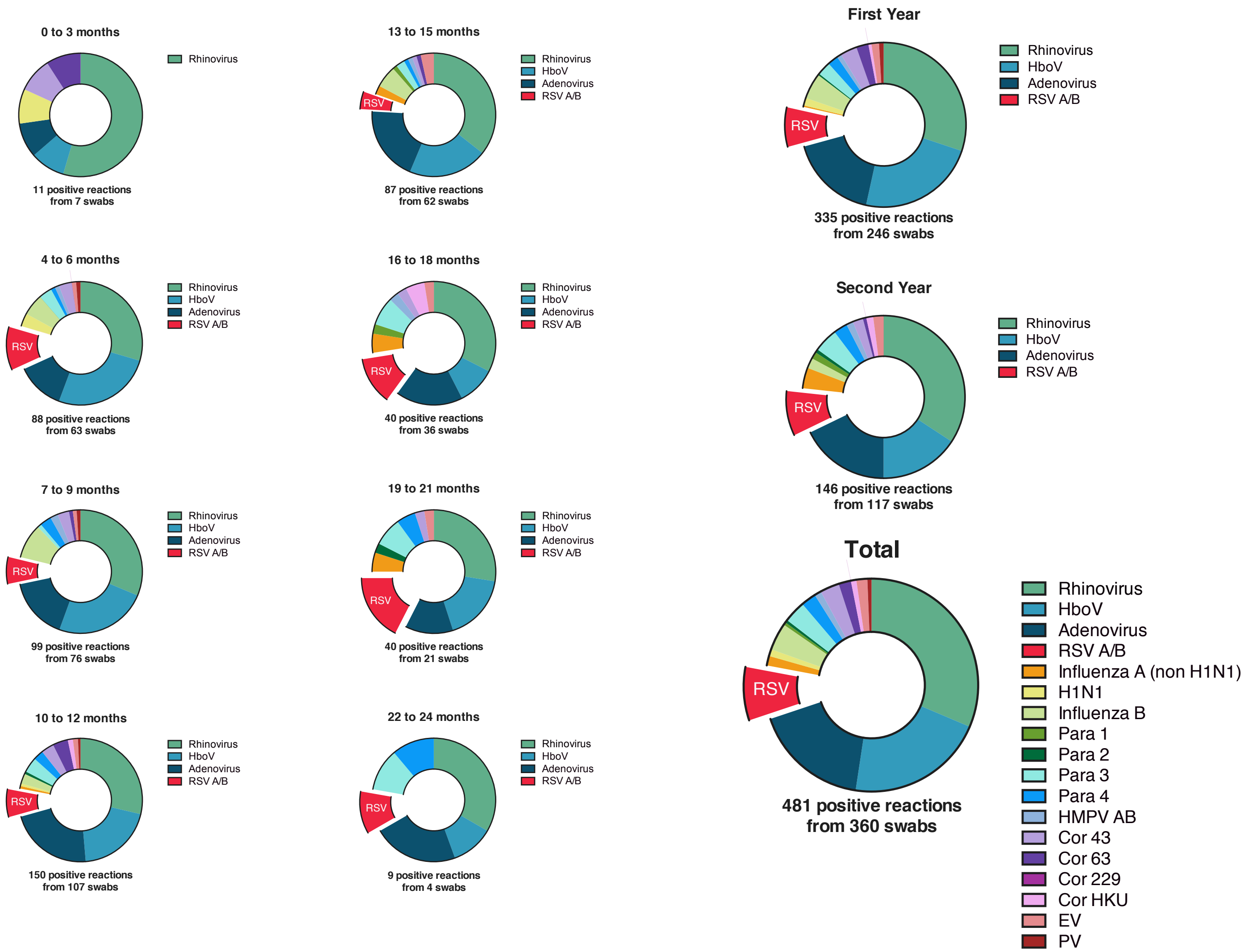


Figure 4.4: Co-infections by pneumonia severity

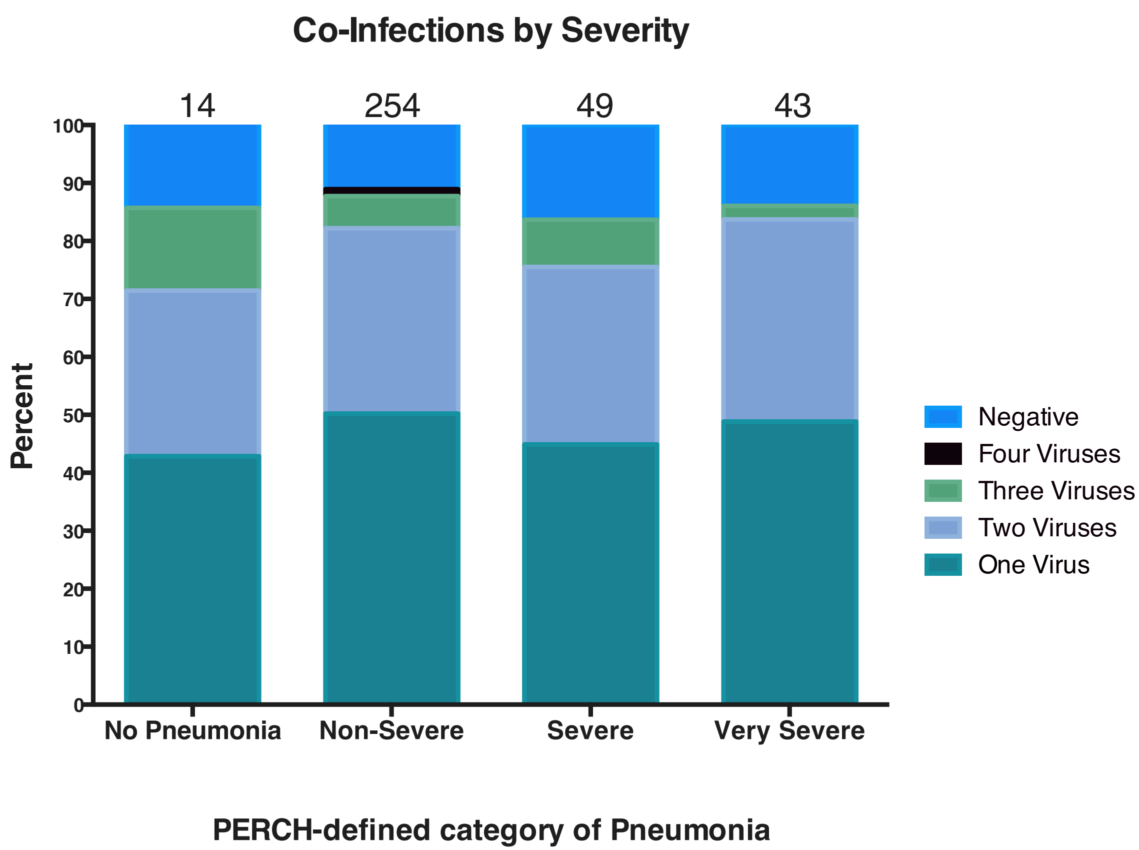


Figure 4.5: Viral detection by pneumonia severity

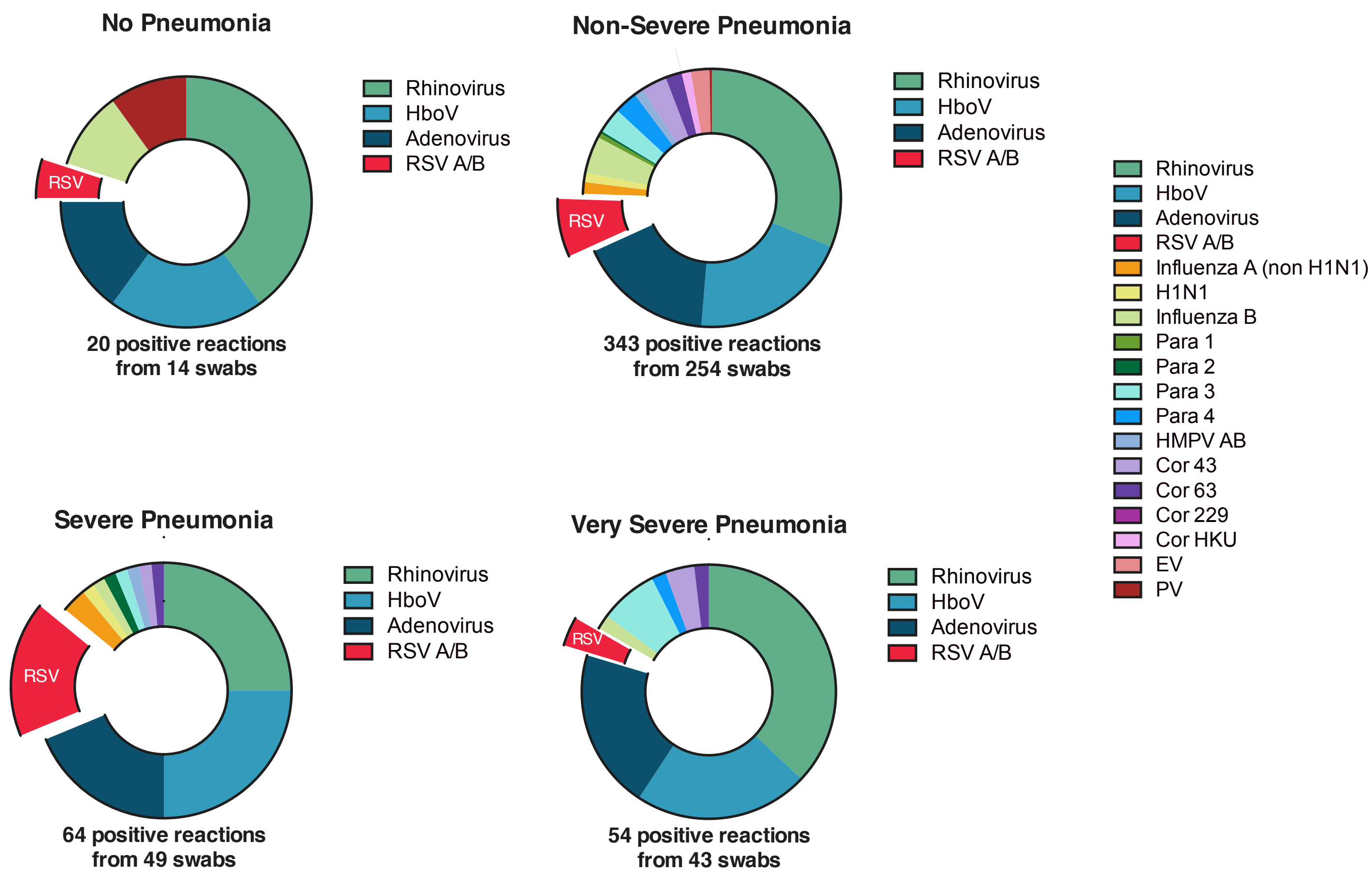


Figure 4.6: Births and NP collection over time

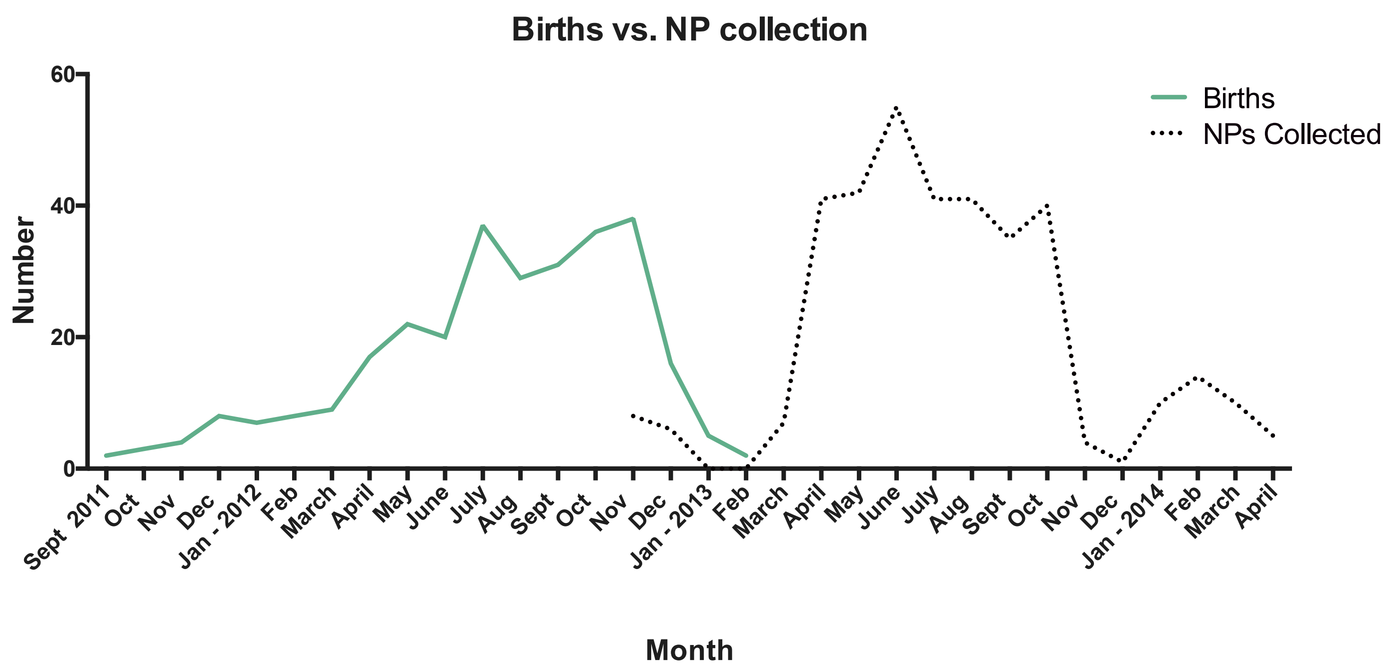


Figure 4.7: Viral detection by month

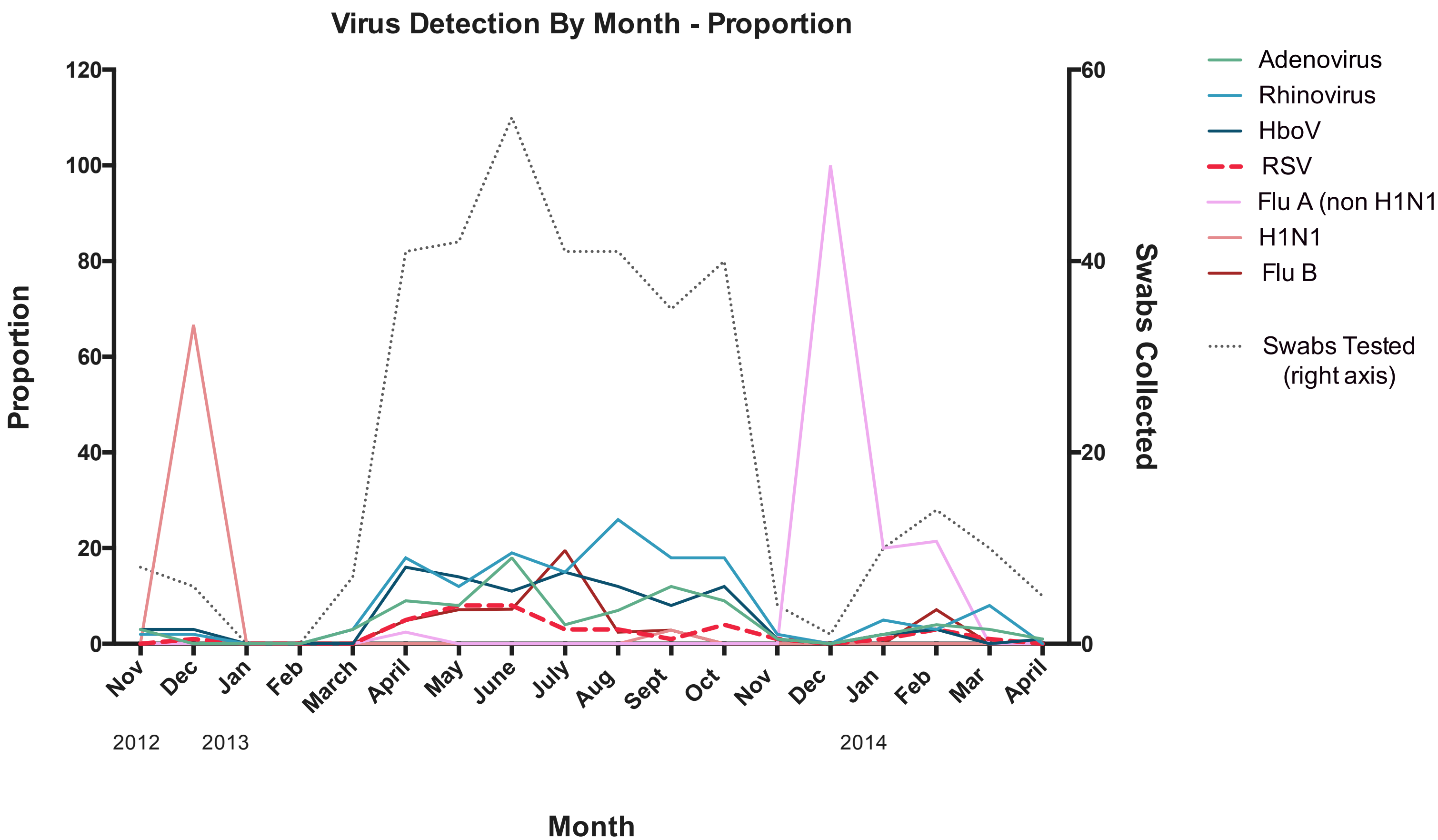


Figure 4.8: Pneumonia by month

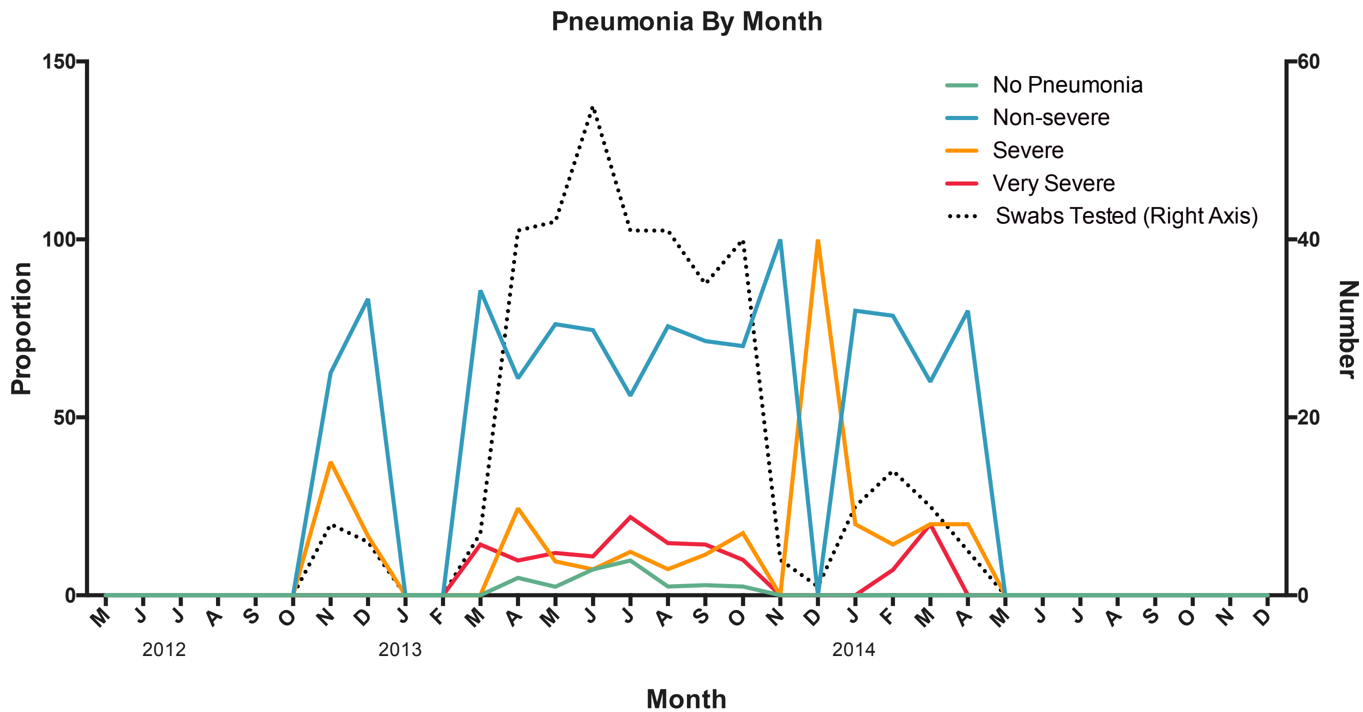
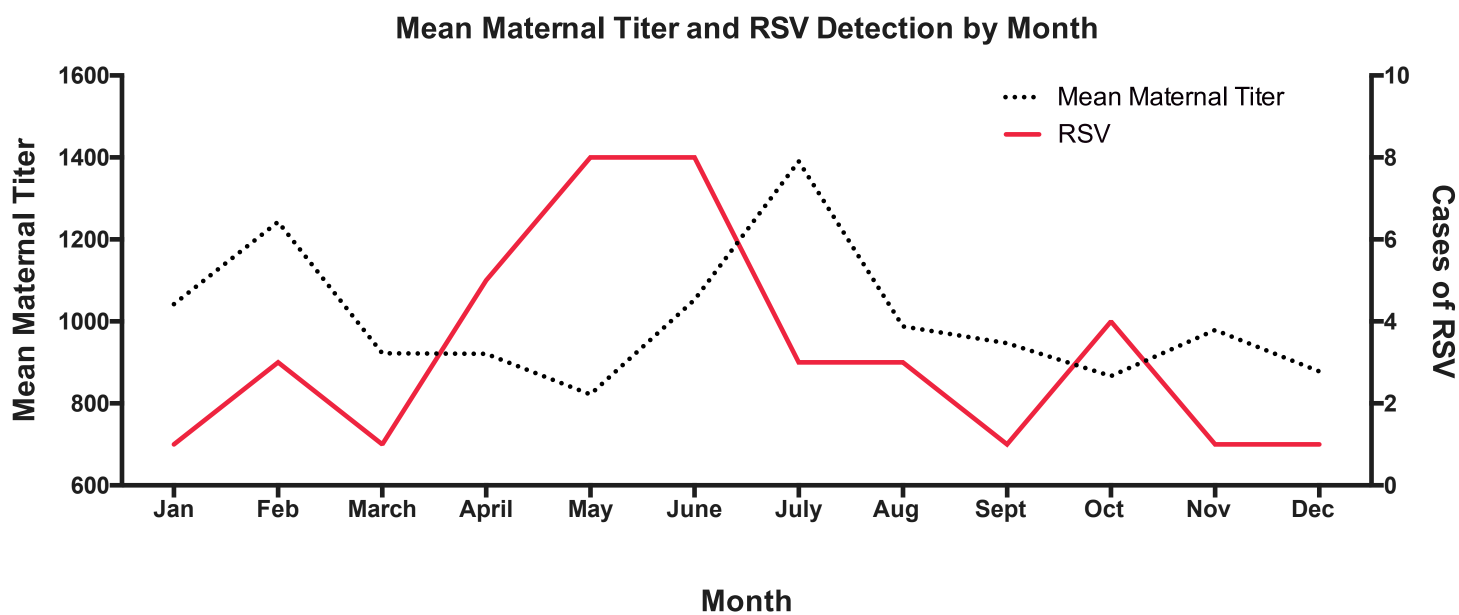


Figure 4.9: Mean maternal RSV PRNT and RSV detection by month



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Chapter 5: Clinical predictors of critical RSV illness in infants and children: data to inform case definitions for efficacy trials

Jessica E. Atwell,[1] Sarah Geoghegan,[2,3] Ruth A. Karron,[1*] Fernando P. Polack[2,4*]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore Maryland, [2]Fundación INFANT, Buenos Aires, Argentina, [3] National Maternity Hospital, Dublin Ireland, [4]Vanderbilt University, Nashville, Tennessee

*both contributed equally to the work

In preparation

5.1 Abstract

Evidence is needed to inform case definitions for clinical endpoints in efficacy trials of respiratory syncytial virus (RSV) vaccines. We analyzed a cohort of 524 infants hospitalized with RSV lower respiratory infection (LRI) in Buenos Aires to determine which clinical signs and symptoms could be used to predict disease severity and identify infants at greatest risk of death. We stratified RSV LRI cases as either non-critical (not requiring oxygen therapy or receiving oxygen via cannula only) or critical (requiring assisted ventilation) and found that oxygen saturation measured via pulse oximetry (SpO_2) of ≤90, fast breathing, and tachycardia were associated with increased odds of critical RSV LRI [adjusted odds ratio (OR) for SpO_2 ≤90: 2.30 (95% CI: 1.26 – 4.24); p: 0.007; for fast breathing: 2.22 (95% CI: 1.19 – 4.16); p: 0.012; for tachycardia 2.35 (95% CI: 1.22-4.50; p: 0.010)]. These findings may assist in the design of upcoming vaccine trials.

Highlights:

- Oxygen saturation of ≤90 measured via pulse oximetry, fast breathing and tachycardia are each associated with increased odds of critical RSV LRI in a cohort of infants in Buenos Aires, Argentina
- Fever (≥38°C) is associated with reduced odds of critical RSV LRI.
- These findings may be useful in the development of case definitions for efficacy trials of RSV vaccines.

5.2 Introduction

Respiratory syncytial virus (RSV) is the leading viral cause of acute lower respiratory illness (LRI) in infants and children.[1] The global burden of RSV LRI in children under 5 years was estimated to be 33.8 million cases in 2005 (95% CI 19.3-46.2).[2] Global estimates of RSV-associated mortality range from 66,000 to 234,000[2, 3] and new estimates are forthcoming.

Substantial progress is being made in efforts to develop RSV vaccines.[4] As of early 2016, there are more than 50 candidate vaccines and 12 in clinical development, including one maternal immunization candidate (NCT02624947) that began phase III testing in 2015.[4, 5] For infants and children, both passive (maternal vaccines; prolonged half-life monoclonal antibody [mAb]) and active immunization strategies are being pursued. The primary goal will be to protect against RSV-associated LRI, given that sterilizing immunity is not expected.[1, 6, 7] However, RSV-associated LRI can vary in severity and outcome: many children will recover spontaneously without intervention, some will recover with oxygen therapy, while others will recover only with assisted ventilation. In countries with constrained resources, the capacity to provide any form of assisted ventilation may be absent or extremely limited; therefore, children with RSV disease severe enough to require this intervention would be more likely to die than children with less severe disease.

In March 2015, the World Health Organization convened an RSV vaccine consultation to provide guidance on vaccine development pathways and on endpoints for clinical trials that would be appropriate and relevant for low and middle-income countries (LMICs).[8] Guidance was provided based upon expert opinion, but it was noted that additional epidemiologic data would be useful to inform case definitions and distinguish between RSV LRI that was non life-threatening ("severe LRI") and RSV LRI that was life-threatening ("very severe LRI").[8]

To this end, we analyzed data from a multicenter hospital-based study of the burden of RSV disease in children in Buenos Aires to assess whether particular signs and symptoms could help distinguish between these disease states, and furthermore, could identify children at greatest risk of death from RSV LRI in LMICs. We developed case definitions for what we termed "non-critical RSV illness" (not requiring oxygen at all, or receiving oxygen via a nasal cannula only) and "critical RSV illness" (requiring assisted ventilation via a mask, CPAP/BiPAP or mechanical ventilation), as children requiring assisted ventilation in LMICs would be at greater risk of fatality. We then analyzed the predictive value of clinical signs and symptoms for development of either outcome.

5.3 Materials and methods

We analyzed data from 1591 infants and children hospitalized with respiratory illness in Buenos Aires in 2013.[9, 10] Children ages 0-24 months were evaluated when admitted to 1 of

13 participating hospitals in Buenos Aires. Demographic, medical history, and physical examination data including oxygen saturation measured by pulse oximetry (SpO_2) at the time of presentation were included in the study database.

Infants who arrived at the hospital not already receiving supplemental oxygen and who were RSV positive by real-time polymerase chain reaction (RT-PCR)[9] were included in this study. In addition, measurement of SpO_2 on ambient air, respiratory rate (RR), evaluation of indrawing at admission were required for inclusion. (**Figure 5.1**)

Children were classified as having critical or non-critical RSV LRI. We defined critical RSV LRI as receiving oxygen via a mechanism more invasive than a nasal cannula (mask, CPAP/BiPAP, or mechanical ventilation). Non-critical cases either did not require supplemental oxygen, or required oxygen via nasal cannula only.

We used WHO guidance for the diagnosis and management of pneumonia to categorize fast breathing by age: 0 to 59 days, ≥60 breaths per minute; >2 months to up to 12 months, ≥50; >12 months to 5 years, ≥40.[11] Tachycardia was defined in beats per minute as: 0-3 months, >160, 3-6 months, >150, >6-12 months, >130, >1-3 years, >125.[12]

Statistical analyses were conducted using Stata 14 (College Station, Texas). Differences in means and proportions were evaluated with Student's t-tests and χ^2 respectively. Receiver operator curve (ROC) analyses were conducted to determine a maximally predictive cut point for SpO_2 based on Youden's index.[13]

Clinical signs and symptoms including SpO2, fast breathing, tachycardia, fever (≥38°C), lower chest wall indrawing (LCWI), wheeze, nasal flaring, and cough were evaluated via univariate and multivariate logistic regression for associations with critical and non-critical RSV LRI. Variables with p-value of <0.1 in univariate analyses or identified as epidemiologically relevant *a priori* (age, weight-for-age, and sex) were included in multivariate models.

5.4 Results

Baseline characteristics

846 of the 1591 children were positive for RSV by PCR; of these, 524 met the additional inclusion and exclusion criteria (**Figure 5.1**). 74 and 450 met our case definitions for critical and non-critical RSV disease, respectively. 513 of the 524 children (98%) required oxygen therapy.

The 524 children in our final subset were representative of all children admitted in 2013 in terms of baseline and demographic characteristics as outlined in **Table 5.1**, however there were statistically significant differences at the time of hospitalization. The mean age at hospitalization of children in the analyzed subset was slightly older than the mean among all children in 2013 (6.2 months vs. 5.4) and children in the subset were more likely to experience tachypnea, LCWI, wheezing, and cough when evaluated at hospital admission.

Children with non-critical and critical RSV LRI were similar across a variety of demographic, epidemiologic, and clinical variables including the proportion male, mean gestational age, birth weight, history of wheezing, congenital heart disease, neurologic conditions, second hand smoke exposure, and household size (**Table 5.2**). However, children with critical RSV LRI were significantly less likely to have an asthmatic father and to have running water or sewage system at home (**Table 5.2**). At hospital admission children with critical RSV were less likely to be febrile, and more likely to show signs of tachypnea, tachycardia and wheezing than children with non-critical RSV. All three deaths were among children with critical RSV.

SpO_2 thresholds

ROC analyses of continuous SpO_2 measurements determined the most predictive cut point to be $SpO_2 \leq 90$. While sensitivity for critical RSV LRI at this cut point is 77%, the corresponding specificity and area under the curve (AUC) are 41% and 0.59 respectively.

Logistic Regression

In univariate logistic regression, $SpO_2 \leq 90$, fast breathing, and tachycardia were statistically significantly associated with increased odds of critical RSV, while fever was associated with decreased odds of critical RSV (Table 2). No other clinical signs and symptoms including LCWI, nasal flaring, cough, or wheeze were statistically significantly associated with critical disease.

In multivariate logistic regression $SpO_2 \leq 90$, fast breathing and tachycardia remained statistically significantly associated with an increased odds of critical disease [adjusted OR for $SpO_2 \leq 90$: 2.30 (95% CI: 1.26 – 4.24); p: 0.007; adjusted OR for fast breathing: 2.22 (95% CI: 1.19 – 4.16); p: 0.012; adjusted OR for tachycardia: 2.35 (95% CI: 1.22-4.50) p: 0.010]. Fever remained associated with reduced odds of critical disease [adjusted OR: 0.31 (95% CI: 0.15 – 0.61); p:0.001). Age, underweight for age, and sex were included in the model due established epidemiologic importance, but were not statistically significantly associated with RSV LRI severity.

We examined these data for evidence of effect modification, however we did not find statistically significant interaction between any of the variables included in these models, or when stratifying by age at 3 and 6 months. However, there was suggestion of potential interaction between fever and fast breathing.

5.5 Discussion

RSV infection causes serious LRI in children worldwide, but is disproportionately associated with life-threatening disease in LMICs.[14] As phase 3 trials of RSV vaccines and mAbs are initiated, it will be important to determine efficacy not only against any RSV LRI, but also against life-threatening ("critical") RSV LRI. In this study of hospitalized children in Buenos Aires, we used assisted ventilation as a proxy for critical RSV LRI and assessed the predictive value of a broad array of relevant signs and symptoms. We found that $SpO_2 \leq 90$, fast breathing, and tachycardia at hospital admission were each positively associated with increased odds of critical RSV LRI, while fever was associated with reduced odds, controlling for age, weight for age, and sex.

These findings are consistent with expert opinion provided at a WHO consultation on RSV vaccines.[8, 11] The SpO_2 cut point of ≤90 is not surprising, given the steep inflection of the oxygen-hemoglobin dissociation curve around that value. However, our study also showed that SpO_2 alone is a relatively nonspecific indicator of critical RSV disease, as many children with non-critical illness also had SpO_2 at or below this value. **(Figure 5.2)** It may be that a composite score that includes this variable in some combination with tachycardia and fast breathing will be helpful; additional studies are needed. We did not find significant associations between critical disease and LCWI, nasal flaring, cough or wheeze as these signs and symptoms were seen across a majority of infants with critical and non-critical RSV in this study. Notably, approximately one-quarter of infants in both groups lacked LCWI, a sign often integral to case definitions of RSV LRI.

Our study was subject to a number of limitations. We focused entirely on children hospitalized with RSV LRI, nearly all of whom required oxygen supplementation. Thus, we were not able to assess the utility of signs and symptoms in the case definition of non-life threatening RSV LRI, since we had no mild RSV illness comparator. In addition, we did not collect data on ability to drink or level of consciousness, both of which were included in the proposed WHO case definition and have previously been included in case definitions for very severe pneumonia.[8, 11] The apparent protective association with fever could be explained by co-infection with other viruses more commonly associated with fever (e.g. influenza), but these data were also unavailable. Importantly, we chose to limit this analysis to children with data available on respiratory rate, oxygen saturation and presence and type of indrawing as those were key variables in our case definitions (**Figure 5.1**). Review of medical records and discussion with study staff informed us that those data were sometimes only recorded if noted to be irregular (for example, respiratory rate might only be written on the chart if the child was determined to be tachypneic by the clinician examining the child). These differences are evident in Table 5.1 and affect the representativeness of our subset for all children with RSV, and furthermore, likely bias our subset to include children with more severe disease.

Similar analyses should be conducted in additional LMICs to confirm and extend these findings across various settings, and within populations that do not have the biases in data collection described above. Larger cohorts are needed to properly evaluate effect modification (particularly between respiratory rate and fever). Additional cut points for SpO_2 may be needed in higher altitudes, and presence of co-infection should be evaluated. If SpO_2 cut points and respiratory rates are to be used in the classification of RSV LRI severity in the context of RSV vaccine trials, it will be essential to standardize equipment and training for pulse oximetry and measurement of respiratory rate across study sites. This investment in training and resources will be essential for determining the impact of these novel vaccines on RSV illness in infants and children.

5.6 References

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5.7 Tables and Figures

Table 5.1: Baseline characteristics of infants and children hospitalized with RSV: All 2013 cases and analysis subset			
	All RSV+ in 2013	RSV+ Subset	P-value
N	846	524	
Male; n(%)	489 (58)	308 (59)	
Gestational age; mean (range)	38 (25-43)	38 (25–42)	
Birth weight in g; mean (range)	3196 (715-8730)	3172 (715–4650)	
IUGR; n(%)	31 (3.7)	22 (4)	
Admitted to NICU when born; n(%)	163 (19)	101 (19)	
Vaccines up to date; n(%)	398 (47)	257 (49)	
Previous episodes of wheeze; n(%)	145 (17)	95 (18)	
Congenital heart disease; n(%)	19 (2.3)	11 (2)	
Down Syndrome; n(%)	5 (0.6)	3 (0.6)	
Neurologic condition; n(%)	9 (1)	6 (1.2)	
Asthma - mother; n(%)	35 (4)	24 (4.6)	
Asthma - father; n(%)	46 (5)	33 (6.3)	
Previous admission to the hospital; n(%)	192 (23)	121 (23)	
Mother smokes cigarettes; n(%)	174 (21)	107 (20)	
Father smokes cigarettes; n(%)	365 (43)	231 (44)	
Mother completed secondary school; n(%)	169 (20)	110 (21)	
Father completed secondary school; n(%)	161 (19)	103 (20)	
Number of siblings; mean(range)	1.8 (0-12)	1.7 (0-10)	
Household size; mean(range)	5.4 (1-21)	5.4 (1-21)	
Dirt floor at home; n(%)	18 (2)	12 (2.3)	
Running water in the home; n(%)	627 (74)	404 (77)	
Sewage system at home; n(%)	301 (36)	195 (37)	
Heating at home: wood/kerosene; n(%)	12 (1.4)	7 (1.3)	
At Hospitalization			
Age in months (mean, range)	5.4 (0-24)	6.2 (0-24)	0.02
Fever when admitted; n(%)	266 (31)	166 (32)	
Tachypnea n(%)	402 (48)	313 (60)	0.005
Tachycardia; n(%)	349 (59)	284 (54)	
Indrawing- any; n(%)	725 (86)	482 (92)	<0.001
Lower chest wall indrawing; n(%)	515 (61)	361 (69)	0.001
Wheezing; n(%)	589 (70)	299 (76)	0.001
Nasal Flaring; n(%)	54 (6.4)	32 (6)	
Cough; n(%)	534 (63)	359 (69)	0.0027
Apnea; n(%)	11 (1.3)	7 (1.3)	
Arrived ventilated; n(%)	112 (13)	n/a	
Required O_2 during admission; n(%)	813 (96)	513 (98)	0.02
Cannula[Ŧ]; n(%)	659 (78)	439 (84)	<0.001
Deaths; n(%)	4 (0.5)	3 (0.6)	

Table 5.2: Baseline characteristics of infants and children hospitalized with RSV: non-critical and critical cases

	Non-critical hospitalized RSV	Critical hospitalized RSV	P-value
N	450	74	
Male; n(%)	267 (59)	41 (55)	
Gestational age; mean (range)	38 (25-42)	38 (31-42)	
Birth weight in g; mean (range)	3180 (715-4650)	3121 (800-4550)	
IUGR; n(%)	19 (4)	3 (4)	
Admitted to NICU when born; n(%)	89 (20)	12 (16)	
Vaccines up to date; n(%)	221 (49)	36 (49)	
Previous episodes of wheeze; n(%)	83 (18)	12 (16)	
Congenital heart disease; n(%)	9 (2)	2 (2.7)	
Down Syndrome; n(%)	1 (0.2)	2 (2.7)	0.009
Neurologic condition; n(%)	5 (1.0)	1 (1.4)	
Asthma - mother; n(%)	23 (5)	1 (1.4)	
Asthma - father; n(%)	32 (7)	1 (1.4)	0.05
Previous admission to the hospital; n(%)	104 (23)	17 (23)	
Mother smokes cigarettes; n(%)	90 (20)	17 (23)	
Father smokes cigarettes; n(%)	198 (44)	33 (45)	
Mother completed secondary school; n(%)	97 (22)	13 (17)	
Father completed secondary school; n(%)	82 (18)	21 (28)	0.04
Number of siblings; mean(range)	1.6 (0-10)	2.3 (0-9)	0.004
Household size; mean(range)	5.3 (1-21)	5.7 (2-15)	
Dirt floor at home; n(%)	11 (2)	1 (1.4)	
Running water in the home; n(%)	354 (79)	50 (68)	0.024
Sewage system at home; n(%)	181 (40)	14 (19)	0.0004
Heating at home: wood/kerosene; n(%)	6 (1)	1 (1.4)	
At Hospitalization			
Age in months (mean, range)	6.2 (0-24)	6.2 (1-24)	
Fever when admitted; n(%)	154 (34)	12 (16)	0.002
Tachypnea n(%)	255 (57)	58 (78)	0.0004
Tachycardia; n(%)	229 (51)	55 (74)	0.004
Indrawing- any; n(%)	410 (91)	72 (97)	[0.07]
Lower chest wall indrawing; n(%)	305 (68)	56 (76)	
Wheezing; n(%)	337 (75)	62 (84)	
Nasal Flaring; n(%)	28 (6)	4 (5)	
Cough; n(%)	307 (68)	52 (70)	
Apnea; n(%)	6 (1)	1 (1.4)	
Arrived ventilated; n(%)	n/a	n/a	
Required O_2 during admission; n(%)	439 (98)	74 (100)	
Cannula[Ŧ]; n(%)	439 (98)	n/a	
Deaths; n(%)	0	3 (4)	<0.001

Table 5.3 Potential markers for and contributors to critical RSV LRI

	Univariate Logistic Regression			Multivariate Logistic Regression		
Variable	**Unadjusted OR**	**95% CI**	**P-value**	**Adjusted OR**	**95% CI**	**P-value**
SpO_2 ≤90 at admission	2.29	1.30 – 4.08	**0.004**	2.30	1.26 – 4.24	**0.007**
Fast Breathing	2.77	1.54 – 4.97	**0.001**	2.22	1.19 – 4.16	**0.012**
Tachycardia	2.29	1.30 – 4.05	**0.003**	2.35	1.22 – 4.50	**0.010**
Fever (≥38°C)	0.37	0.19 – 0.71	**0.003**	0.31	0.15 – 0.61	**0.001**
Subcostal indrawing	1.11	0.61 – 2.03	0.727	n/a		
Wheeze	1.73	0.90 – 3.33	0.099	n/a		
Nasal flaring	0.86	0.29 – 2.53	0.786	n/a		
Cough	1.10	0.64 – 1.88	0.725	n/a		
Age in months	0.99	0.95 – 1.05	0.952	0.96	0.90 – 1.02	0.196
Sex	0.85	0.52 – 1.40	0.525	0.93	0.55 – 1.58	0.78
Underweight	1.01	0.48 – 2.16	0.968	1.02	0.46 – 2.26	0.962

Tachypnea: 0-2 months: respiratory rate (RR) ≥60 breaths per minute, >2-12 months: RR ≥50, >12 months: RR ≥40
Tachycardia: 0-3 months: heart rate (HR) in beats per minute >160, >3-6 months: HR >150, >6-12 months: >130, 1-3 years: HR >125
Underweight: Weight for age Z-score ≤2
Sex: reference is male

Figure 5.1 Inclusion and exclusion criteria

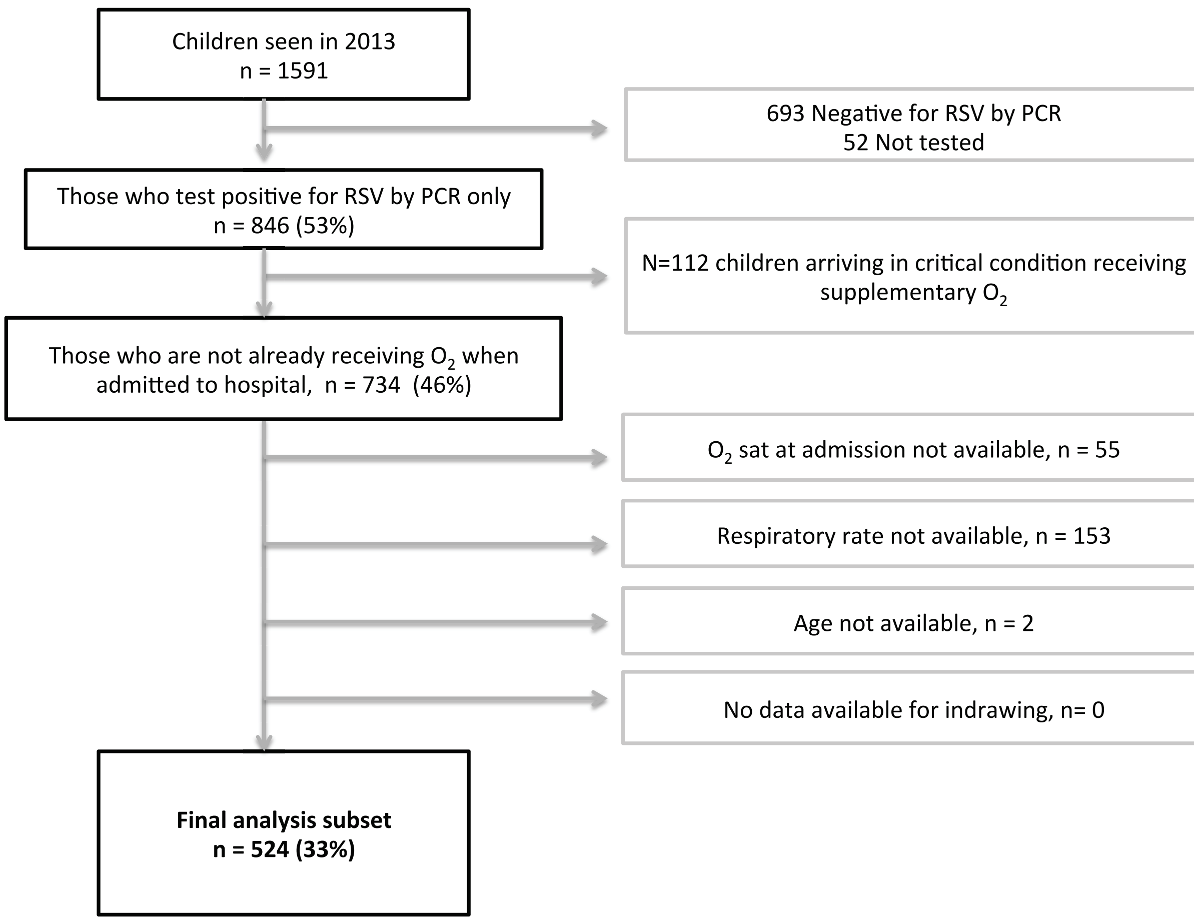
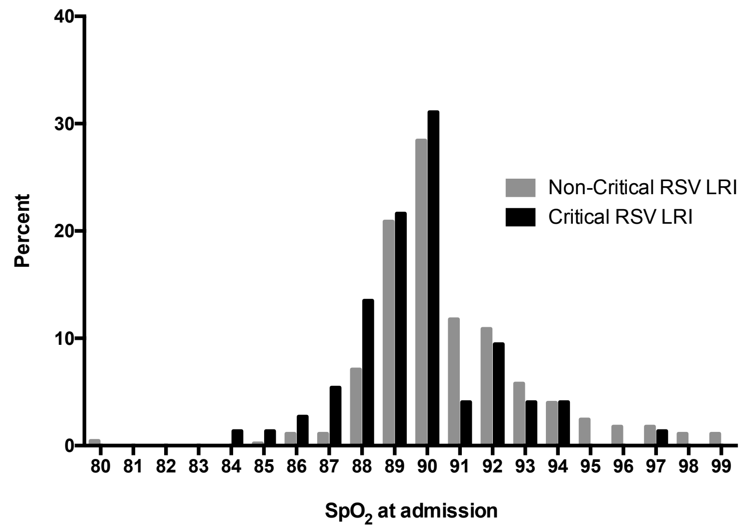


Figure 5.2: SpO_2 at admission for children hospitalized with critical or non-critical RSV LRI



Chapter 6: Discussion

6.1 Summary of study findings

After more than fifty years, RSV vaccine development has entered a very exciting phase. A number of vaccines for active and passive protection of infants against RSV disease are in clinical trials, and one or more of these vaccines may be available in the not-too-distant future. Findings described in this thesis will be useful in addressing questions related to RSV vaccine evaluation and implementation, particularly in low and middle-income settings.

6.1.1 Objective 1: Barriers to transplacental transfer of RSV antibodies

The goal of this objective was to evaluate transplacental transfer of RSV antibody between mothers and their full term infants in Papua New Guinea, and the associations between placental malaria or hypergammaglobulinemia and impairment of that transfer. We found that approximately one third of full-term mother-infant pairs demonstrated impaired transplacental transfer of RSV antibody and that hypergammaglobulinemia, but not placental malaria, was associated with this impairment. We also demonstrated that approximately one third of infants were born with antibody titers below the level believed to be protective against RSV LRI (RSV PRNT of <1:200).

6.1.2 Objective 2: Characterization of viral LRI in Papua New Guinea

The aim of this objective was to evaluate the etiology and seasonality of viral LRI, particularly RSV LRI, in the same coastal region of Papua New Guinea, where the burden of pneumonia is high, but little data are available on the pathogens associated with respiratory disease. Our results demonstrate a significant, year-round burden of pneumonia and frequent detection of respiratory viruses in the nasopharynx of children under two years old with LRI. The most common viruses detected were rhinovirus, adenovirus, bocavirus and RSV, and co-infections with these pathogens were common. RSV was the only virus statistically significantly associated with severe pneumonia, and despite our inability to sample the youngest infants with presumably the highest burden of RSV LRI, our study indicates that RSV may be present in more half of the observed cases of severe pneumonia in this population (PAF 56%). Furthermore, RSV was observed throughout the first two years of life, demonstrating its importance in older infants and young children. Our ability to properly evaluate seasonality was limited, but RSV was detected most frequently in March, April and May, and no other viruses demonstrated strong seasonal patterns. Interestingly, RSV antibody titers among pregnant women in this same population are lowest among those who delivered directly preceding this period of peak RSV detection, and increased substantially afterward.

6.1.3 Objective 3: Case definitions and predictors of severe RSV LRI

This analysis evaluated potential case definitions for critical and non-critical RSV illness that might be useful for efficacy trials of RSV vaccines. We utilized data from infants hospitalized with PCR-confirmed RSV in Buenos Aires, Argentina and the surrounding peri urban area. Critical RSV illness was defined as the need for assisted ventilation (mask, CPAP/BiPAP, or mechanical ventilation). We demonstrated that oxygen saturation of ≤90 measured via pulse oximetry (SpO_2), tachypnea and tachycardia at hospital admission were each associated with increased odds of critical RSV LRI, and that fever (≥38°C) was associated with reduced odds of critical RSV LRI.

6.2 Implications for policy and practice

These findings may be useful across multiple aspects of RSV vaccine evaluation and implementation. RSV is a pathogen of global importance, but deaths from RSV are more likely to occur where supportive care, particularly supplemental oxygen and ventilatory support, are not available. Phase III clinical trials will be conducted in a variety of settings and will include LMICs where vaccination will be essential to reduce RSV-associated morbidity and mortality. For these trials, case definitions for both life-threatening RSV and less severe disease are needed. Preliminary case definitions have been generated using expert opinion,[1] but additional data are needed to inform these definitions and support decision-making and recommendations. Findings from this study and others will be useful in aiding those discussions and in the design of phase III and potentially even phase IV clinical trials of the efficacy and effectiveness of active and passive vaccines for RSV.

Once vaccines are licensed and available for use, governments and public health stakeholders in resource limited settings will need to evaluate prioritization of RSV immunization. However, there is currently a paucity of data on the burden and seasonality of respiratory viruses including RSV in tropical settings, and indications of substantial variation in seasonality between and even within countries.[2] Studies of the etiology of viral LRI in Papua New Guinea are extremely limited, but the burden of respiratory disease is well documented.[3, 4] Our data, although limited in scope, provide important contemporary information on the presence of respiratory viruses, co-infections, and relationships to severe and very severe LRI. These data may be useful in determining how and when vaccines might be evaluated or used in this area, where pneumonia is the leading cause of under five mortality.

Maternal immunization is a very attractive intervention for prevention of RSV in very young infants who are the most vulnerable to RSV LRI.[5, 6] However, the effectiveness of maternal immunization could be diminished by conditions that interfere with or reduce transplacental transfer of maternal antibodies. Pre-term delivery is one known limiting factor of antibody transfer, but the importance of HIV, placental malaria and hypergammaglobulinemia in the impairment of the transfer of antibodies in general, and RSV antibodies in particular, must be better understood before maternal immunization is implemented in areas endemic for these conditions. Although placental malaria has been associated with impairment of transplacental antibody transfer in previous studies, our work indicates that hypergammaglobulinemia, and not placental malaria, is associated with impairment of RSV antibody transfer and suggests that earlier studies noting associations between impairment and placental malaria may have been confounded by hypergammaglobulinemia. It may be warranted to further research what conditions and chronic infections are upstream of hypergammaglobulinemia, and to determine appropriate prevention or treatment strategies for those conditions among women receiving maternal immunizations. However, our work also demonstrates that a substantial proportion of both mothers and infants have RSV antibody levels below the level correlated with protection in some studies, but that high titers of maternal RSV antibody levels can mitigate the effect of impairment from hypergammaglobulinemia. The absolute amount of antibody in the cord blood is more important than the ratio of cord to maternal titers (CMTR) in terms of protection from disease. For this reason, sufficient boosting of maternal antibody levels via maternal immunization may be able to circumvent impairment. These data, along with the findings from objective 2 about the presence of RSV and its association with severe pneumonia among older children in this population indicate that improved immunity to RSV through both active and passive immunization in Papua New Guinea would be beneficial.

6.3 Strengths and limitations

These studies exhibited a number of strengths and limitations. We were fortunate with objective 1 to be able to study transplacental transfer in two temporally distinct populations in the same region, but before and after substantial regional reductions in malaria. We demonstrated the same level of impairment in both the early (Alexishafen) and late (FIS) time points, as well as a lack of association between placental malaria and the presence of an association with hypergammaglobulinemia. While not a true counterfactual, these populations afforded us a unique and valuable opportunity to evaluate these relationships in very similar groups of women and their infants separated by only a few years, but experiencing very different levels of exposure to malaria. Furthermore, we were able to evaluate malaria and hypergammaglobulinemia separately, which other studies had either not done or not been able to do, as the majority of women experienced both conditions.

Nesting our aims within larger cohort studies among pregnant women and their infants, as well as making use of stored specimens retrospectively, enabled to us leverage the investment in both time and resources designed to study other questions. However, doing so meant we were limited by the follow up time points and specimen collection optimized for the parent study. We were also unable to provide input into the baseline data collected at study enrollment. The negative impacts of nesting are most detrimental to our ability to study seasonality, as we did not begin collecting NP swabs until late in follow up, many children were well into their second year of life, limiting our ability to understand the etiology of LRI in the youngest infants. Furthermore, we were unable to collect specimens from two full calendar years, which would have been helpful in understanding temporal trends.

6.4 Future research and next steps

It will be important to confirm and extend our findings relating to placental malaria, hypergammaglobulinemia and impairment of RSV antibody transfer, both in PNG and elsewhere, before maternal immunization for RSV is widely implemented. We are in the process of analyzing 325 additional mother-infant pairs from births occurring between 2010 and 2012 in the same regions of Madang Province, PNG. Preliminary analyses indicate consistency with our previous findings. Significant impairment of RSV antibody transfer is observed within approximately one third of pairs in this study, and as before, impairment is associated with hypergammaglobulinemia, but not placental malaria in multivariate logistic regression. We were able to demonstrate these relationships using a high throughput RSV microneutralization antibody assay, suggesting that this finding is independent of the method used for measurement of RSV neutralizing antibody.

We had hoped to be able to study both the decay of maternally derived RSV antibody in infants, as well as infant response to routine childhood immunizations in this population but were unable to do so. Unfortunately, we had very few sequential 1, 3 and 6-month blood specimens available from infants for whom we also had cord blood due to prioritization of resources within the parent study. Studies of this nature would still be valuable in this setting and elsewhere. Other groups have evaluated decay of maternal RSV antibodies among infants,[7] but additional data will be helpful to inform maternal immunization programs. Given that our data indicate high maternal RSV titers can override impairment of transfer due to hypergammaglobulinemia or other conditions, mathematical modeling techniques that can take into account impairment and decay may help with the creation of guidelines for necessary titer thresholds to be achieved by vaccines for maternal immunization.

We had also hoped to evaluate cord blood titers at birth and protection from RSV LRI in the first three months of life in the context of objective 2, but very few specimens were collected among children in this age group for the reasons described previously. Such data would be valuable in understanding the ability of passively acquired antibody to protect infants from severe disease. This will likely be a primary endpoint of the ongoing and upcoming phase III trials, but is important to understand these relationships with naturally derived antibody as well.

In objective 1, we found that mean maternal RSV titers were lower among women with placental malaria compared to women without, although these findings were only statistically significant for the Alexishafen cohort (GMT 220 vs. 303, p=0.02) and not for the FIS cohort (229 vs. 246, p=0.81). Our ongoing work to understand placental malaria and antibody transfer confirmed this finding (maternal GMT in placental malaria positive mothers: 586 vs. 736 in those without placental malaria, p=0.01). We did not have a clear biologic mechanism to explain this phenomenon. However, objective 2 demonstrated that mean maternal titer was lowest in the calendar months directly preceding peak detection of RSV in infants. It seems these two factors are related. Interestingly, while malaria transmission is perennial in PNG, the majority of births among women with placental malaria in our studies happened to occur between March and September (largely because of temporal trends in enrollment). Therefore, we hypothesize that the association we saw with reduced mean RSV titer among women with placental malaria might actually be tied to a temporal association with the RSV season, and not associated with malaria. This requires further study. Furthermore, studies optimized to evaluate seasonality and true burden of both LRI and RSV in this setting are warranted.

Our analysis of the hospitalized cohort in Argentina will be helpful in providing data to guide experts in their recommendations for case definitions and endpoints for RSV clinical trials, but similar analyses should be conducted in a variety of LMIC settings. There were important sources of bias (both selection and information bias) in our cohort, as variables included in our inclusion criteria were more likely to be collected among infants with severe disease. Ideally, analyses similar to ours would be conducted among different populations of infants, both in terms of geography and severity of RSV infection to evaluate whether the case definitions and endpoints proposed are valuable and appropriate. Furthermore, creation of composite scores with better predictive value, sensitivity and specificity may be ideal.

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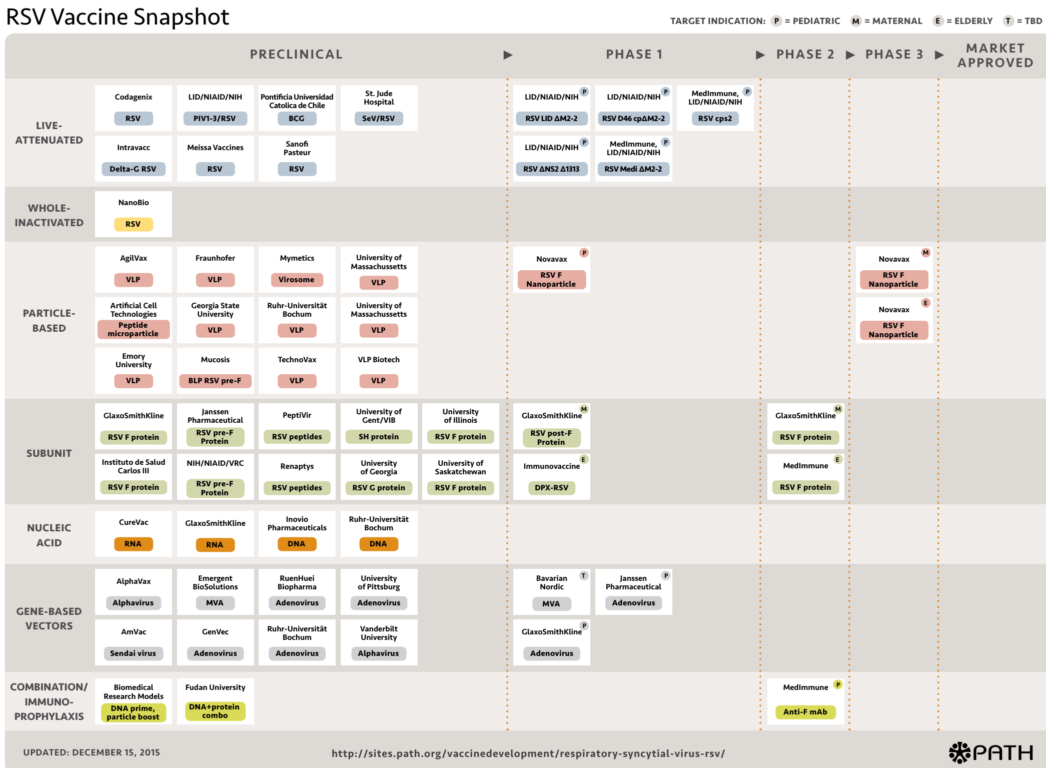
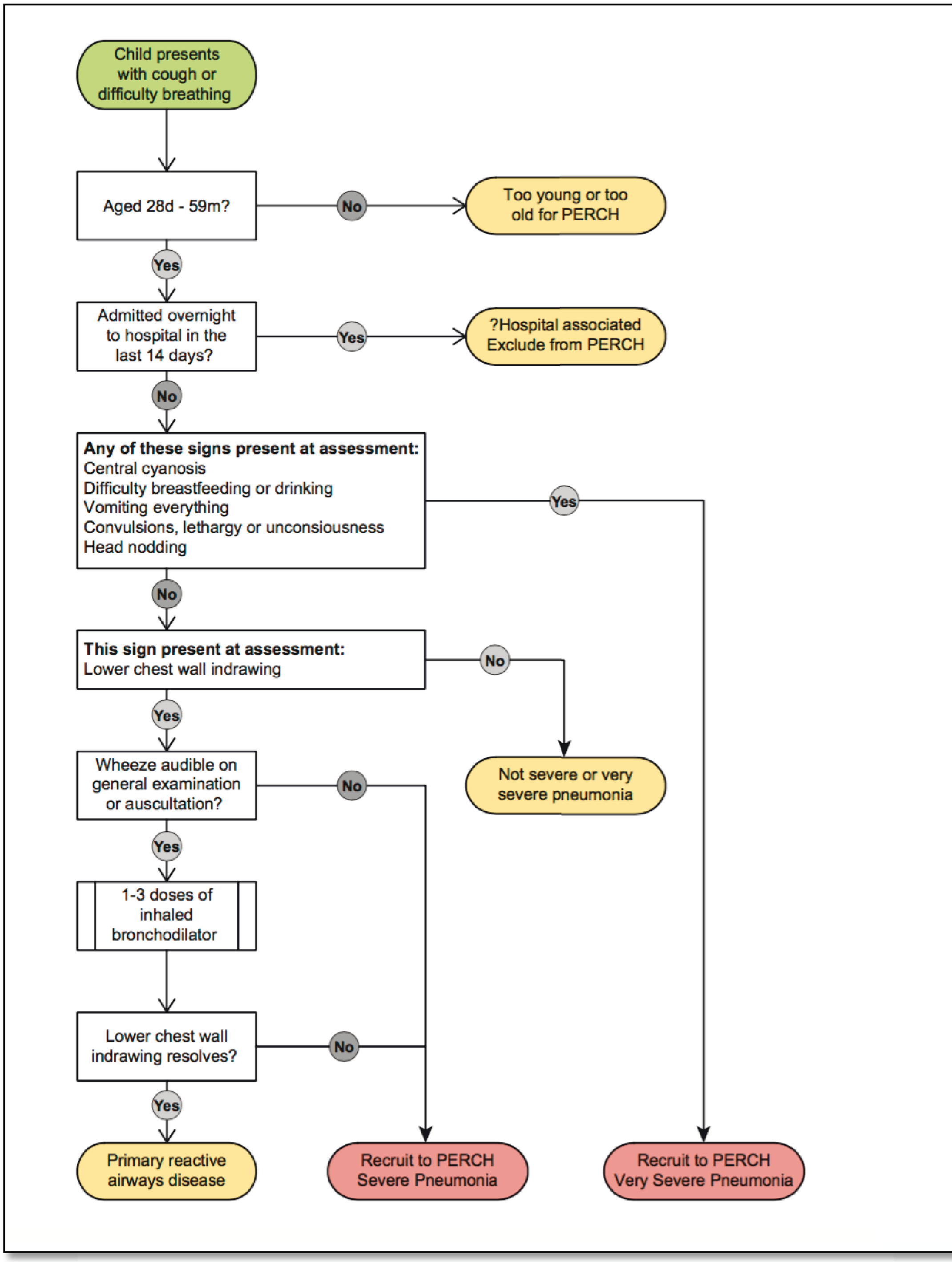
Appendices

Appendix 1: RSV Vaccine Snapshot



Appendix 2: Case Definitions of Pneumonia

From The PERCH study, Scott et al, CID 2013



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newborns residing in the Gambia and in The United States. Clinical and diagnostic laboratory immunology. 1996;3(4):477-9.

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CIRRICULUM VITAE

Jessica Erin Atwell

Born August 31, 1982 in Oceanside, CA, USA

PERSONAL DATA

Department of International Health
Johns Hopkins Bloomberg School of Public Health
615 North Wolfe St.
Baltimore, MD 21205-2179
805.705.0119
Jatwell5@jhu.edu

EDUCATION AND TRAINING

Expected March 2016	Doctor of Philosophy, International Health Global Disease Epidemiology and Control Johns Hopkins Bloomberg School of Public Health Baltimore, MD Advisor: Dr. Ruth Karron Thesis project: *Respiratory syncytial virus (RSV) vaccines on the horizon: data to inform clinical trials and implementation*
May 2011	Master of Public Health Concentration: Infectious Diseases Johns Hopkins Bloomberg School of Public Health Baltimore, MD Advisor: Dr. Neal Halsey Capstone project: *Pertussis in California, 2005-2010: Epidemiology and Geographic Distribution of Cases and Non-Medical Vaccination Exemptions*
May 2011	Certificate Vaccine Science and Policy Johns Hopkins Bloomberg School of Public Health Baltimore, MD
June 2005	Bachelor of Science Molecular, Cellular, and Developmental Biology University of California, Santa Barbara

	Santa Barbara, CA
June 2005	Bachelor of Arts English University of California, Santa Barbara Santa Barbara, CA
January 2003 – July 2003	Study Abroad La Trobe University Bundoora, Victoria Australia

PROFESSIONAL EXPERIENCE

Position:	**Pre-doctoral Investigator**
Institution:	PI: Dr. Ruth Karron, Center for Immunization Research Department of International Health Johns Hopkins Bloomberg School of Public Health Baltimore, MD
Dates:	July 2012 – Current
Principal Responsibilities:	

- Study transplacental transfer of naturally acquired respiratory syncytial virus (RSV) antibodies in women with placental malaria and hypergammaglobulinemia in Papua New Guinea (laboratory work and data analysis)
- Study seasonality and distribution of viral causes of lower respiratory tract infections in infants in Papua New Guinea (laboratory work and data analysis)
- Conduct comparative analyses of assays for detection and quantification of RSV antibodies (laboratory work and data analysis)
- Evaluate data from infants hospitalized with RSV in Argentina to determine case definitions for and clinical predictors of critical (life-threatening) RSV lower respiratory infection

Position:	**Graduate Student Researcher**
Institution:	PIs: Drs. Alain Labrique and Kenrad Nelson Departments of International Health and Epidemiology Johns Hopkins Bloomberg School of Public Health Baltimore, MD

Dates: May 2012 – September 2012

Principal Responsibilities:

- Composed a comprehensive landscape analysis on Hepatitis E virus for the Bill and Melinda Gates Foundation in collaboration with internal and external experts
- Prepared a proposal for a phase III clinical trial to study the safety and efficacy of an investigational Hepatitis E vaccine for use in pregnant women in Bangladesh
- Contributed to a review on the fetal and neonatal health consequences of vertically transmitted Hepatitis E virus infection

Position: **Graduate Student Researcher**

Institution: Dr. Neal Halsey
Department of International Health
Johns Hopkins Bloomberg School of Public Health
Baltimore, MD

Dates: August 2011 – May 2012

Principal Responsibilities:

- Conducted spatial and temporal clustering analysis of pertussis cases and nonmedical exemptions to immunization in California using SaTScan software

Position: **Intern**

Institution: Drs. Deblina Datta and Peter Hansen
Policy and Performance Team, Monitoring and Evaluation Group
Gavi (The Vaccine Alliance); formerly the Global Alliance for Vaccines and Immunization (GAVI)
Geneva, Switzerland

Dates: June 2011 – August 2011

Principal Responsibilities:

- Focused on identifying flaws in and revising methods used by Gavi and partners to evaluate and improve country administrative data systems and estimates of immunization coverage
- Conducted a literature review and critical evaluation of existing data quality assessment methods used by Gavi and other organizations

- Compiled data from Gavi countries regarding administrative systems, and analyzed agreement (or lack thereof) between various estimates of vaccine coverage for Gavi countries
- Modeled future deaths averted for non-pentavalent Hepatitis B-containing vaccines
- Assisted with pre-HPV vaccine rollout activities within the Secretariat

Position: **Graduate Student Researcher**

Institution: PI: Dr. Hope Johnson
International Vaccine Access Center (IVAC)
Johns Hopkins Bloomberg School of Public Health
Baltimore, MD

Dates: July 2010 – December 2010

Principal Responsibilities:

- Assisted with a systematic literature review to estimate adult global disease burden and distribution of meningococcal and invasive pneumococcal disease.

Position: **Public Health Microbiologist**

Institution: Microbial Diseases Laboratory
California Department of Public Health
Richmond, CA

Dates: August 2007 – June 2010

Principal responsibilities:

- Conducted molecular epidemiology on vaccine preventable diseases (primarily meningococcal disease, pneumococcal disease and pertussis)
- Coordinated efforts between the Microbial Diseases Laboratory and the epidemiologists in the Immunization Branch of the California Department of Public Health
- Contributed to CDPH and CDC infectious disease surveillance efforts
- Developed new diagnostic and research assays with particular emphasis on rapid detection and strain typing of bacterial pathogens
- Led the laboratory component of a WHO twinning project between CDPH and the Ethiopian Health and Nutrition Research Institute (EHNRI) to introduce diagnostic and

research capability at the Ethiopian national public health lab for meningococcal disease

- Implemented a project to characterize bacterial isolates of *S. pneumoniae* from patients with suspected pneumococcal vaccine failure as part of surveillance on post-Prevnar® invasive serotypes of *S. pneumoniae* in California
- Conducted characterization of bacterial isolates (*S. pneumoniae, S. aureus, S. pyogenes*) from patients with co-infections of 2009 H1N1 Influenza A for local and national surveillance
- Position required frequent written and verbal communication with local hospitals, public health laboratories and disease control officers as well as program staff and epidemiologists at the state and national levels
- Created and maintained of a variety of databases, protocols, and documents
- Routinely assisted senior staff with compilation of data and the editing and preparation of scientific articles for publication or presentation

Position: **Public Health Microbiologist**

Institution: Microbial Diseases Laboratory
California Department of Public Health
Richmond, CA

Dates: November 2005 – August 2007

Principal responsibilities:

- Performed molecular epidemiology in the form of Pulsed-Field Gel Electrophoresis on food-borne bacterial pathogens for CDC's PulseNet Program—the national molecular subtyping network for food-borne disease surveillance.
- Investigated clusters and outbreaks of *E. coli*, salmonella, listeriosis, and shigelosis
- Developed and pilot tested new molecular typing assays for the characterization of bacterial pathogens

PROFESSIONAL ACTIVITIES

Society Membership

American Society of Tropical Medicine & Hygiene (2011 – current)
American Society for Microbiology (2005 – 2011)

Consultations

Gavi (The Vaccine Alliance), Geneva, Switzerland
Monitoring and Evaluation Team, Policy and Performance Group
August 2012 – January 2013

- Impact modeling for Hepatitis B-containing vaccines across all Gavi countries from 2000 to 2020 as part of the 2012-2013 Decade of Vaccines collaboration.

PATH, Ferney-Voltaire, France
Project OPTIMIZE
December 2011 – February 2012

- Assisted project OPTIMIZE staff in preparation of a report for the Bill and Melinda Gates Foundation assessing country cold chain capacities
- Focused on identifying potential bottlenecks for all countries planning to introduce pneumococcal and/or rotavirus vaccines with support from Gavi in 2011-2015.

Gavi (The Vaccine Alliance), Geneva, Switzerland
Monitoring and Evaluation Team, Policy and Performance Group
August 2011 – January 2012

- Assisted secretariat with design and drafting of a new tool for immunization data quality assessment.
- Conducted impact modeling for Hepatitis B-containing vaccines across all Gavi countries from 2000 to 2020 as part of 2011-2012 Decade of Vaccines collaboration.

Working Groups

Health and Economic Benefits Working Group, 2012-2013 Decade of Vaccines Collaboration that contributed to the development of the Global Vaccine Action Plan

Members: Jessica Atwell (Johns Hopkins University), Dagna Constenla (Johns Hopkins University), S. Deblina Datta (Gavi), Ingrid Friberg (Johns Hopkins University), Marta Gacic-Dobo (WHO), Sue Goldie (Harvard School of Public Health), Peter Hansen (Gavi), Lisa Lee (Gavi), Orin Levine (Johns Hopkins University), Meredith O'Shea (Harvard School of Public Health), Sachiko Ozawa (Johns Hopkins University), Susan Reef (CDC), Meghan Stack (Johns Hopkins University), Peter Strebel (WHO), Chutima Suraratdecha (PATH), Steven Sweet (Harvard School of Public Health), Yvonne Tam (Johns Hopkins University), Emilia Vynnycky (Health Protection Agency), Damian Walker (Bill & Melinda Gates Foundation), Neff Walker (Johns Hopkins University), Steve Wiersma (WHO)

EDITIORIAL ACTIVITIES

Peer Review Activities

Pediatrics (4)
2013 - Current

HONORS AND AWARDS

2015	The Clements-Mann Fellowship in Vaccine Sciences Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2015	The Ludwig Family Award Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2015	Distinguished Student Award Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2015	The David J. Sencer Scholarship EIS Alumni Association Atlanta, GA
2014	First place poster 9th International Respiratory Syncytial Virus Symposium Stellenbosch, South Africa
2014	Young Investigator Award, second tier mention Annual meeting of the American Society of Tropical Medicine & Hygiene (ASTMH) New Orleans, LA
2014	Second place poster Vaccine Day Student Poster Competition Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2013	First place poster Delta Omega Poster Session, GDEC sub-competition Johns Hopkins Bloomberg School of Public Health Baltimore, MD

2012	First place poster Vaccine Day Student Poster Competition Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2013	The Clements-Mann Fellowship in Vaccine Sciences Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2011	Travel Fellowship (to support internship at Gavi) Center for Immunization Research Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2011	MPH Capstone Award for Outstanding Achievement Johns Hopkins Bloomberg School of Public Health Baltimore, MD
2007	Superior Performance Award California Department of Public Health Richmond, CA
2005	UCSB Student Volunteer Appreciation Award University of California, Santa Barbara Santa Barbara, CA
2005	Dean's List University of California, Santa Barbara Santa Barbara, CA
2004	Most Appreciated Volunteer 2004 Santa Barbara AIDS Walk Santa Barbara, CA
2000	UC Regents Academic Scholarship University of California, Santa Barbara Santa Barbara, CA

PUBLICATIONS

Journal Articles

1. **Atwell, J.E.**, Thumar, B., Robinson, L.J., Tobby, R., Yambo, P., Ome-Kaius, M., Siba, P.M., Unger, H.W., Rogerson, S.J., King, C.L., Karron, R.A. Impact of placental malaria and hypergammaglobulinemia on transplacental transfer of respiratory syncytial virus antibody in Papua New Guinea. JID. 2015. doi:10.1093/infdis/jiv401

2. Krain, L.J., **Atwell, J.E.**, Nelson, K.E., Labrique, A.B. Review Article: Fetal and neonatal health consequences of vertically transmitted hepatitis E virus infection. Am J Trop Med Hyg. 2014:90(2) 365-70

3. **Atwell, J.E.,** Van Otterloo, J., Zipprich, J., Winter, K., Harriman, K., Salmon, D.A., Halsey, N.A., Omer, S.B. Nonmedical vaccine exemptions and pertussis in California, 2010. Pediatrics. 2013 Sep 30:132(4) 624-30.

4. Richards, J.L., Wagenaar, B., Van Otterloo, J., Gondalia, R., **Atwell, J.E.**, Kleinbaum, D., Lee, T., Salmon, D.A., Omer, S.B. Nonmedical exemptions to immunization requirements in California: a 16-year longitudinal analysis of trends and associated community factors. Vaccine. 2013. June 24:31(29):3009-13.

5. Lee, L.A., Franzel, L., **Atwell, J.**, Datta, D., Friberg, I.K., Goldie, S.J., Reef, S.E., Schwalbe, N., Simons, E., Strebel, P.M., Sweet, S., Suraratdesch, C., Tam, Y., Vynnycky, E., Walker, N., Walker, D.G., Hansen P.M. The estimated mortality impact of vaccinations forecasted to be administered during 2011-2020 in 73 countries supported by the GAVI Alliance. Vaccine. 2013 Apr:31:B61-B72.

6. Materna, B., Harriman, K., Rosenberg, J., Shusterman, D. Windham, G., **Atwell, J.**, Beckman, S., Mortensen, E., Zipprich, J., Occupational Transmission of Neisseria meningitidis-- California 2009. JAMA. 205(1):29 January, 2011. Reprinted from *Morbidity and Mortality Weekly Report* 2010 Nov 19; *59(45): 1480-83*.

7. Probert W.S., Ely J., Schrader K., **Atwell J.**, Nosoff A., Kwan S. 2008. Identification and Evaluation of New Target Sequences for Specific Detection of *Bordetella pertussis* by Real-Time PCR. J. Clin Microbiol. 2008 Oct:46(10):3228-31

Solicited Commentaries

8. **Atwell, J.E.**, Salmon, D.A. Pertussis resurgence and vaccine uptake: Implications for reducing vaccine hesitancy. Pediatrics. 2014. DOI: 10.1542/peds.2014-1883

CIRRICULUM VITAE

Jessica Erin Atwell

PART II

TEACHING

Classroom Instruction

Vaccine Development and Application (223.622.01)
Department of International Health
Johns Hopkins Bloomberg School of Public Health

Lecture Title: Bacterial Vaccines and Toxoids: Diphtheria, tetanus and pertussis
October 2015, October 2014

Lecture Title: Pertussis
October 2013

Teaching Assistantships

Johns Hopkins Bloomberg School of Public Health
Department of International Health

2012, 2013, 2015	Global Disease Control Programs and Policies (223.680.01) Primary Instructors: Drs. Christian Cole and Alain Labrique Grading TA
2011, 2012, 2013, 2014	Vaccine Development and Application, onsite (223.622.01) Primary Instructors: Drs. Ruth Karron and Laura Hammitt (2013, 2014); Dr. Neal Halsey (2011, 2012)
2011, 2012	Vaccine Development and Application, online (223.622.81) Primary Instructor: Dr. Neal Halsey
2011, 2012	Special Topics in Vaccine Science (223.867.01) Primary Instructor: Dr. Anna Durbin
2011	Introduction to Global Health (undergraduate) Primary Instructor: Dr. Jim Tielsch

Johns Hopkins Bloomberg School of Public Health
Department of Epidemiology

2013, 2014	Fundamentals of Epidemiology, online (550.694.81)

	Primary Instructors: Drs. Marie Diener-West and Sukon Kanchanaraksa
2012, 2013	Principles of Epidemiology, summer term (340.601.01) Primary Instructors: Drs. Rosa Crum and Greg Kirk
2011, 2012, 2013	Principles of Epidemiology, first term (340.601.01) Primary Instructors: Drs. Bill Moss and David Celentano

Departmental Tutoring

2013, 2014, 2015	Principles of Epidemiology Tutor Department of Epidemiology Johns Hopkins School of Public Health

Mentoring

2013	Advisor to: Aaron Chang, Amanda Valledor, Dominic Thomas, Allison Moyer, Jin Ryang Chung, Erik Tan, Paola Donis, Rajiv Deshpande (Undergraduate Students in Biomedical Engineering) Senior design project: Facilitating controlled temperature chain strategies for last mile vaccine transport Johns Hopkins University Baltimore, MD

RESEARCH GRANT PARTICIPATION

Biologic barriers to transplacental antibody transfer, respiratory syncytial virus (RSV) burden, and the relationship between cord blood RSV neutralizing antibody and RSV lower respiratory infection

2014 - 2015
PATH Vaccine Solutions
PI: Dr. Ruth Karron

Objectives:

1. To confirm and extend our group's previous findings concerning transplacental transfer of RSV neutralizing antibody in women and infants living in coastal Papua New Guinea (PNG)
2. To assess the contributions of RSV and other respiratory viruses to acute lower respiratory illness (ALRI) in a birth cohort of infants in coastal PNG
3. To explore the relationship between cord RSV neutralizing antibody titer and RSV ALRI in the first year of life in a birth cohort of infants in coastal PNG

Principal responsibilities as a Graduate Student Investigator: Travel to the field site in Papua New Guinea to support the parent study this project was nested within, and to assist with specimen handling, shipping, data entry, data cleaning and interim analysis. To receive, organize and catalogue specimens in Baltimore, complete laboratory assays, and perform data analysis.

Assessment of the effect of placental malaria on transplacental transport of respiratory syncytial virus (RSV) antibodies in Papua New Guinea

2012 - 2014
PATH Vaccine Solutions
PI: Dr. Ruth Karron

Objectives:

1. To determine the efficiency of transplacental transfer of RSV neutralizing antibody in the context of placental malaria by measuring RSV neutralizing antibody titers in paired maternal and cord sera from two cohorts of women in Papua New Guinea, where both *P. falciparum* and *P. vivax malaria* are prevalent
2. To evaluate the effect of hypergammaglobulinemia on transplacental transfer of RSV antibodies in these same cohorts in PNG
3. To evaluate high-throughput neutralizing antibody assays for RSV to aid in the standardization of RSV antibody measurement in the context of clinical trials for RSV vaccines

Principal responsibilities as a Graduate Student Investigator: Receive, organize and catalogue specimens in Baltimore, complete laboratory assays, and perform data entry and analysis.

PRESENTATIONS

Scientific Meeting Presentations

Impact of maternal malaria and hypergammaglobulinemia on transplacental transfer of RSV neutralizing antibodies in coastal Papua New Guinea

Oral presentation for poster competition finalists (first place)
9th International Respiratory Syncytial Virus Symposium
November 2014
Stellenbosch, South Africa

Impairment of transplacental transfer of antibody

Symposium 79: Maternal Immunization to Prevent Early Infant Respiratory Infections and Sepsis: On the Near Horizon
Annual meeting of the American Society of Tropical Medicine & Hygiene
November 2014
New Orleans, LA

Posters

Impact of maternal malaria and hypergammaglobulinemia on transplacental transfer of RSV neutralizing antibodies in coastal Papua New Guinea. **Atwell, J.E.**, Thumar, B., Robinson, L.J., Tobby, R., Yambo, P., Ome-Kaius, M., Stanisic, D.I., Siba, P.M., Unger, H.W., Rogerson, S.J., King, C.L., Karron, R.A.

Malaria in Pregnancy Symposium
Johns Hopkins Malaria Research Institute
Johns Hopkins School of Public Health
Baltimore, MD
April 2015

Johns Hopkins School of Public Health Delta Omega Poster Competition
Baltimore, MD
February 2015

9th International Respiratory Syncytial Virus Symposium
Stellenbosch, South Africa
November 2014
Poster #91
First Place

Annual meeting of the American Society of Tropical Medicine & Hygiene
New Orleans, LA
November 2014
Poster #1119
Young Investigator Award, second tier mention

Johns Hopkins School of Public Health Vaccine Day
Baltimore, MD
October 2014
Second place

Nonmedical Vaccine Exemptions and Pertussis in California, 2010. **Atwell, J.E.,** Van Otterloo, J., Zipprich, J., Winter, K., Harriman, K., Salmon, D.A., Halsey, N.A., Omer, S.B

Johns Hopkins School of Public Health Delta Omega Poster Competition
February 2013
Baltimore, MD
First place – GDEC sub-competition

Johns Hopkins School of Public Health Vaccine Day
October 2012
Baltimore, MD
First place

GAVI's Immunization data quality assessment project: Developing a toolbox to evaluate country immunization coverage estimates. Atwell, J.E.

Johns Hopkins School of Public Health Vaccine Day
October 2011
Baltimore, MD

Pertussis in California 2005-2010: Epidemiology and geographic distribution of cases and personal belief exemptions. Atwell, J.E., Halsey, N.A.

Johns Hopkins School of Public Health Vaccine Day
October 2011
Baltimore, MD